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A population Analysis of Juniperus in The Missouri River Basin: Taxonomic Interrelationships Between Juniperus scopulorum Sarg. and J. virginiana L. in Missouri River Basin

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Taxonomic Interrelationships Between *Juniperus scopulorum* Sarg. and *J. virginiana* L. in the Missouri River Basin

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Abstract

Juniper has been used since early pioneer days in the protective tree plantings established on the windswept Great Plains. More recently juniper has been used as a basic component of the shelterbelt plantings which, by the thousands, dot the prairies of the Great Plains from Texas to Canada. Research to improve the quality of Plains tree planting materials, including *Juniperus,* is proposed or is now in progress.

In the fall of 1965, 72 native stands of *Juniperus* were sampled throughout the Missouri River Basin—an area encompassing most of the Central and Northern Great Plains. The purpose was to determine the occurrence and extent of variation in this *Juniperus* population and to clarify some of the interrelationships between *Juniperus scopulorum* Sarg. and *J. virginiana* L., thus establishing a sounder foundation upon which to begin improvement of the planting stock with respect to characteristics desirable in Plains forestry.

The study was based on a taxonomic evaluation of 38 gross morphological, foliage, cone and seed, and infrared characters of 675 trees. Separate analyses involved 675 ovulate and staminate trees with 16 characters, 343 ovulate trees with 24 characters, and 142 ovulate trees with 38 characters in common.

Frequency distributions of character values, correlation coefficients of all possible character combinations, hybrid indices and percent germ plasm distributions were determined to characterize the population. IBM 7040 and 360 electronic computers were utilized as an aid in the analysis.

Analyses suggested the *Juniperus* population throughout the Missouri River Basin to be of hybrid derivation with neither parental type being found. A strong tendency toward bimodality within the population was demonstrated, however, indicating the presence of two different species—*J. scopulorum* and *J. virginiana.* The analyses demonstrated an introgressive trend between southeast and northwest over the Basin from the reported range of *J. virginiana* into that of *J. scopulorum.*

A zone of hybrid values comparable to an F_1 type was demonstrated along and adjacent to a line extending from extreme western Kansas, northward into western Nebraska, diagonally northeastward through southwestern South Dakota and the Badlands, then northward into central North Dakota. The trend toward *J. scopulorum* on the west side of this zone was more rapid than was the trend toward *J. virginiana* on the east. Data supporting the hypothesis of movement of *J. scopulorum* germ plasm along the Missouri River into eastern Nebraska and Missouri were obtained. Evidence of introgressive hybridization involving a third species (*J. horizontalis* Moench.) into the *J. scopulorum* population of Montana and northern Wyoming was also obtained.

A quantitative technique utilizing infrared (IR) spectroscopy—original in its application to a population analysis over a broad area—was developed for obtaining chemical data from crude lipid extracts of *Juniperus* cone pulp. High correlation of chemical data with morphological data was demonstrated. Results suggested the desirability of IR analysis as a source of relationship evidence in other investigations.

An interpretation of the data is presented which proposes that the *Juniperus* population of the Missouri River Basin could have evolved through divergence from *J. scopulorum* rather than from introgression between *J. scopulorum* and *J. virginiana*.

Introduction

Juniper has been used since early pioneer days in the protective tree plantings established on the windswept Great Plains. More recently juniper has been used as a basic component of the shelterbelt plantings which, by the thousands, dot the prairies of the Great Plains from Texas to Canada. It has frequently been observed that shelterbelts containing conifers have improved over the years while those not containing conifers have, in general, deteriorated. Engstrom (1940, p. 2–3) stated "... that conifers, particularly the cedars, are the soundest foundation upon which Plains forestry tree planting can be built. Long-life, adaptability to a wide range of site conditions, year-round protection, regeneration, wildlife value, and comparative freedom from rodent, insect, and livestock damage, as well as disease resistance combine to give the cedars a popular and technical appeal which assure them in my opinion, the most prominent spot in future planting operations."

Concepts of planting and design of field shelterbelts have shifted during recent decades from the wide plantings of the 1930's and early 1940's, containing only 10 to 20 percent coniferous species, to narrower belts often consisting entirely of single rows of eastern redcedar (*Juniperus virginiana* L.). In Nebraska and elsewhere throughout the Great Plains region in areas susceptible to wind erosion, these narrower shelterbelts are spaced at 20–30 rod intervals across the fields; and, along with strip-cropping and "stubble-mulch" tillage, are intended to provide year-round protection to the fields and crops.

Preston and Brandon (1946) reported that a good all-purpose protective tree planting for northeastern Colorado would consist of two to six rows of ponderosa pine with an exterior row on each side of Rocky Mountain juniper (*Juniperus scopulorum* Sargent). Thus these native, drought-resistant, and long-lived species truly occupy an eminent position in the list of plant materials used in shelterbelt plantings throughout the Great Plains. This is particularly true in the Central and Northern Great Plains where interest in tree planting to control soil erosion by winter, as well as summer,

winds and to provide crop and animal protection has increased during recent years.

Most of the juniper planting stock used in the shelterbelt plantings in the Central and Northern Great Plains originates from native stands within this region. However, considerable natural variation is present in the Great Plains juniper population—not only throughout the geographic area but within individual stands as well. Although the significance of seed source is now better appreciated, little specific information is on record about the origins of the seed sources for most of the established plantings—that is, exactly where the seed came from, or whether the seed lots were composite or separate seed collections.

The Central and Northern Great Plains include portions of the natural ranges of both Rocky Mountain juniper and eastern redcedar (Sudworth, 1915; and Fowells, 1965). Hybrid swarms between these two species have been reported within the boundaries of past and current seed collecting areas where species ranges meet or overlap (Fassett, 1944b). However, much speculation and misunderstanding about the two species still exist among experienced foresters. For instance, what are the real differences between the species; where are the parental types located; what is the nature of the variation within presumed and reported hybrid swarms; and where are these hybrid swarms located?

Projects designed to improve the genetic constitution of Great Plains tree planting materials are proposed and/or are in progress, including those for juniper. To proceed effectively toward this objective, however, we must have an adequate understanding of the extent and nature of the genetic variation of this juniper population as it exists in nature. A taxonomic investigation of the juniper population within the Central and Northern Great Plains (encompassed within a well defined, natural drainage system—the Missouri River Basin) would provide much-needed information about some of the variability within the genus and pinpoint the locations of that variability. Such knowledge would be of help to seed collecting agencies and nurserymen and would provide a sounder basis for improving *Juniperus* planting materials for use in shelterbelts, recreational plantings, and highway beautification projects throughout the Great Plains and elsewhere.

The objectives of this study were, therefore:

1. to determine the occurrence and extent of variation in morphological characters of foliage, cones and seed of *Juniperus scopulorum* Sarg. and *Juniperus virginiana* L. within the

Missouri River Basin, with special attention to major river drainages and to the reported and suspected areas of species overlap and/or abutment in the Central and Northern Great Plains; and

2. using the information obtained in objective 1, to clarify the interrelationships between the two species within the above geographic region.

Review of Literature

Classification

Chamberlain (1935) classified the genus *Juniperus* as follows:

Division.....SPERMATOPHYTA
Sub-division.....GYMNOSPERMAE
Order.....CONIFERAE
Family.....CUPRESSACEAE
Genus.....JUNIPERUS

Other treatments of the genus are given by Sudworth (1915), Gates (1928), Sargent (1933), Van Dersal (1938), Rehder (1940), Fassett (1945c), Preston (1947), Fernald (1950), Pool (1951), Phillips *et al* (1953), Dallimore and Jackson (1961) and others.

Endlicher (1847) created three sections within the genus *Juniperus: Caryocedrus* (woody, cupressoid-like cones), *Oxycedrus* (fleshy berry-cones), and *Sabina* (acicular-type leaves to reduced scale-type leaves). Of the three sections, *Sabina* is the largest containing about thirty species. The population investigated in this study are members of the *Sabina* section.

Many workers have considered the Missouri River Basin juniper population to consist of two discrete or separable species. Hall (1952a) for example, constructed a key to the native species of the *Sabina* section applicable to species found in the Great Plains region. He separated the two species studied as follows:

(Extracted from key, p. 6.)

A. Leaves with entire margins.
  3. Leaves overlapping; tips acute; glands oval or rarely elliptic and shorter than the distance from the gland to the leaf tip.
    4. Upright tree; fruit blue-black, less than 6.5 mm. in diameter.
      5. Leaves 3–4 mm. long; fruit 3.5–6 mm. in diameter, very slightly longer than wide.....*J. virginiana*

3. Leaves not overlapping, tips obtuse, glands elliptic or rarely oval and longer than the distance from the gland to the leaf tip ..... *J. scopulorum*

HISTORY

Eastern redcedar has been recognized since colonial days for its durability, cutting qualities, rich color, and aroma (Williamson, 1957). It is said that Louisiana's capital, Baton Rouge, took its name from this tree—the name being given to the tree by early French colonists (U.S. Forest Service, 1955). Harrar (1957) cited several references to the species by the English colonists as early as 1632, and in 1749 by Benjamin Franklin. Linnaeus (1753) named the species *Juniperus virginiana* apparently from specimens found in the English colony of Virginia.

Rocky Mountain juniper was long considered to be a western form of eastern redcedar, the principal difference being that the former required two years for seed maturity while the latter required only one year. Rocky Mountain juniper, then called mountain redcedar, was first discovered by Lewis and Clark in 1804 (Sudworth, 1915). Sudworth explained that the first technical name given the tree was *Juniperus excelsa* Pursh in 1814 (preoccupied).

Between 1838 and 1897 it was called *J. virginiana, J. occidentalis,* and *J. virginiana* var. *montana.* Sargent (1897) distinguished the tree from *J. virginiana* in 1897 on the basis of fruit size, number of years to seed maturation, and bark characteristics; and named it *J. scopulorum.*

DISTRIBUTION

Juniperus is widely distributed over the northern hemisphere from the Arctic Zone to the mountains of the Tropics. Hall (1947) stated that about 40 species are recognized. Harlow and Harrar (1937) reported that 13 species are native to the United States.

The distribution of *Juniperus virginiana* (Fig. 1) is reported from southern Maine, southern Ontario, southern Wisconsin, southern Minnesota, most of the eastern half of South Dakota, extending into the southwestern corner of North Dakota; then southward through the eastern half of Nebraska, most of Kansas and Oklahoma (excluding the Panhandle) into northwestern Texas. The extent of the southward extension is not clearly established, but it is in most of Arkansas, the northern portions of Mississippi, Alabama, Georgia, South Carolina to the Atlantic Coast, and northward again to southern Maine (Williamson, 1957; and Fowells, 1965).

Juniperus scopulorum (Fig. 1) is reported throughout the drier,

lower mountains and foothills of British Columbia and Alberta, Canada; south through the Great Basin, Rocky Mountain and Northern Plains States to New Mexico and northern Arizona (Sudworth, 1908, 1915; and Fowells, 1965). Its distribution in the Northern Plains States, however, is limited to the western portions of North Dakota, Nebraska, and northeastern Colorado (Herman, 1958). Distribution maps for one or both species are illustrated by Sudworth (1915), Fassett (1945c), Little (1949), Pool (1951), Hall (1952a), Williamson (1957), Herman (1958), and Fowells (1965).

Habitat, Soils, and Timber Types

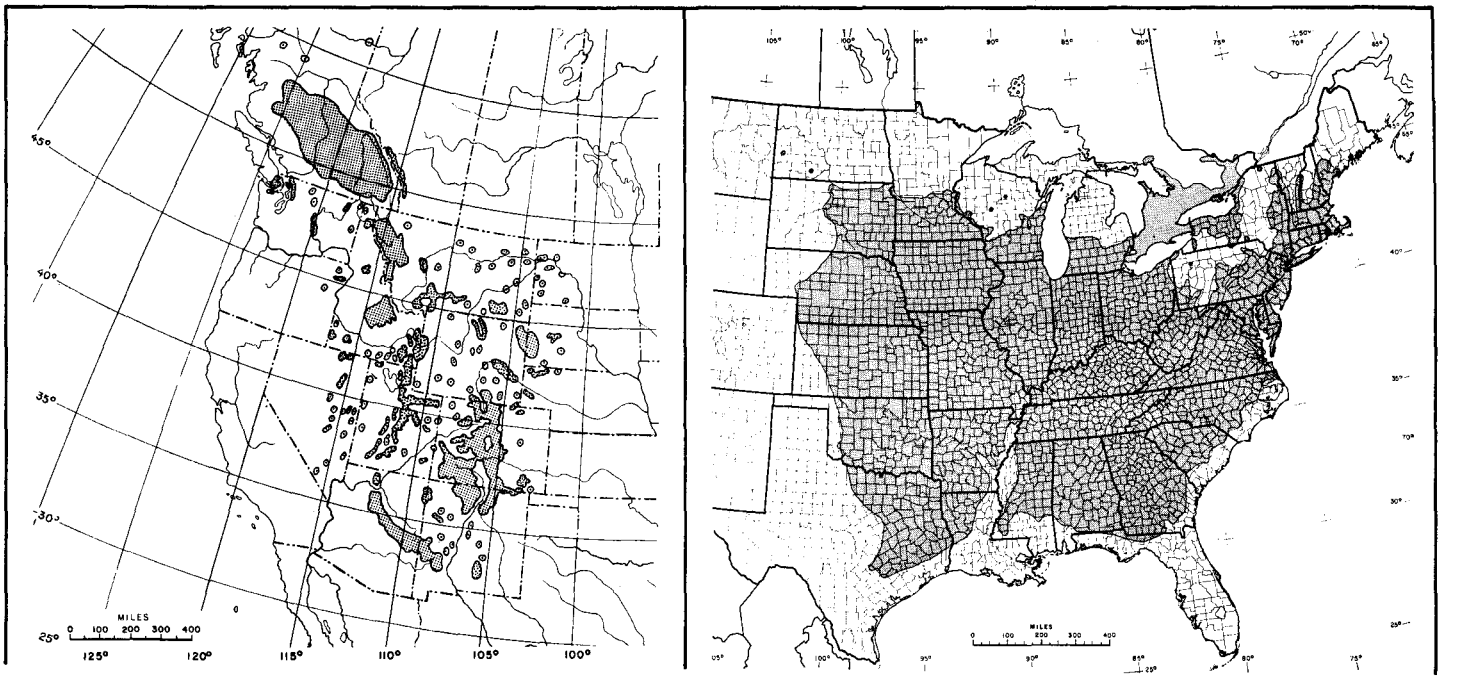
Eastern redcedar grows under a wide range of climatic conditions as evidenced by its wide distribution. Rainfall varies from 16 to 60 inches within its natural range. Average temperatures vary considerably over its range, and the growing season varies from 120 to 250 days (Fowells, 1965). Eastern redcedar grows on a wide variety of soils ranging from dry rock outcrops to swampy land. Albertson (1940) and Hall (1952a) reported it often found on thin soils with limestone and dolomite outcrops or other dry, rocky sites. Afanasiev (1949) and Arend and Collins (1949) concluded that depth of soil and drainage are the most important site factors affecting growth. Natural stands of eastern redcedar grow in soils that vary widely in pH from 4.7 to 7.8 (Arend, 1950).

Eastern redcedar is found on ridgetops, slopes, and flat land, and often grows on dry, exposed sites and abandoned fields (Fowells, 1965). In the western part of its range, the species is found on the north-facing slopes and along streambanks where it is protected from heat and drought (Albertson, 1940). In the east (Missouri, Arkansas, Kentucky, Tennessee, Alabama, etc.) eastern redcedar frequently grows in rocky limestone and dolomite areas (Harper, 1912; Collingwood, 1938; and Read, 1952).

Lundegardh (1957) stated that it has been known for 100 years that certain plants occur exclusively in, or at least prefer, calcium soils. He explained that, in nature, calcium can acquire significance for the propagation of these plants in that calcium ions partially remove the poisonous effects (cation exchange) of hydrogen ions and/or that calcium serves as a trace element.

Fowells (1965) reported that eastern redcedar occurs mainly in the following timber types:

Type 46	Eastern redcedar (pure)
Type 47	Eastern redcedar-Pine
Type 48	Eastern redcedar-Hardwood
Type 49	Eastern redcedar-Pine-Hardwood



Juniperus scopulorum Sarg.

Juniperus virginiana L.

FIG. 1.—The botanical ranges of Rocky Mountain juniper (*Juniperus scopulorum* Sarg.) and eastern redcedar (*Juniperus virginiana* L.); after Fowells, 1965.

Rocky Mountain juniper generally occupies the dry, subhumid regions (Thornthwaite, 1948). Price and Evans (1937) concluded that the species can endure temperature extremes of –35° to +100°F but does best where average minimums are less than –10° to –5°F, where July temperatures range from 60° to 75°F, and average January temperatures range from 15° to 40°F. Fowells (1965) reported that the growing season, when the mean temperature is above 32°F, varies from 140 days at high elevations to 230 days near sea level. Average annual precipitation varies within its range from 12 inches in the Southwest Great Basin and eastern slope of the Rocky Mountains in Colorado to 26 inches in the Puget Sound area.

While these are the data available from Weather Bureau records, it must be realized that mean temperature and mean precipitation figures are not really significant to plant survival. It is the extremes rather than the means which determine whether a plant survives or succumbs. For instance, the mean annual temperature in two widely separated regions (A and B) is 70°F. However, in region A where the temperature fluctuates from –40°F to +120°F during the year, an entirely different flora would be supported than in region B, where a temperature of 20°F to 90°F is maintained. Similar effects would result if extreme fluctuations in the distribution of precipitation in one area were compared to another region having a uniform distribution of the same total amount of precipitation.

Scott (1929) reported that Rocky Mountain juniper also grows best on calcareous and somewhat alkaline soils. Members of the species are found on soils derived from basalt, limestone, sandstone, and shale. They grow on open exposed bluffs, rocky points, and southern exposures where most trees cannot exist (Longyear, 1925), but do best along ravines, in sheltered canyons, and other protected areas (Sudworth, 1915, and Thornthwaite, 1948).

Rocky Mountain juniper is found principally in two forest cover types:

Type 220	Rocky Mountain juniper
Type 239	Pinyon-Juniper

Taxonomic Studies

The distribution of *Juniperus* is extensive, ranging over most of the United States. In earlier times, relationships between the junipers of Montana, for instance, were obscure from those found further east in Virginia. Without critical comparative studies it is understandable that the whole population, or at least large segments of it, was considered a single species. However, as observational reports gave way to increasingly critical population studies, the

tendency increased to assign specific status to segments of the larger population. Engelmann (1877) considered *J. scopulorum* and *J. virginiana* to be one species; and Dallimore and Jackson (1961) listed *J. scopulorum* as a variety of *J. virginiana.* The apparent majority of other current workers, however, consider them to be separate species.

Fassett (1944a) found three factors which makes the differentiation of Rocky Mountain juniper and eastern redcedar difficult. First, the variation within either one of the species is often more conspicuous but never as constant as the difference between them. He found that the variation within any colony of either species is often as great as within the species as a whole. Second, the specific characters are highly variable and necessitate the use of statistical analysis in differentiating the species. Third, the two species may occur together in a colony; and hybrid forms may or may not be found.

Hall (1947) acknowledged a taxonomic relationship between eastern redcedar and Rocky Mountain juniper but stated that the species are sometimes difficult to distinguish. He stated that in regions where the two species meet, it is necessary to turn to quantitative characters since there apparently are no qualitative characters which separate them. Hall (1952b) stated that the study of the *J. scopulorum-J. virginiana* complex is, indeed, one of the most difficult problems in American junipers because the two species are so similar.

Fassett (1944b) reported on presumed hybrid swarms of *J. virginiana* and *J. scopulorum.* He based his findings on 16 sampling sites (six in Kansas, four in Nebraska, four in South Dakota, and two in North Dakota). He concluded that: (1) where each species grows by itself, each retains pure specific characters; (2) in the western part of the *J. virginiana* range certain tendencies toward *J. scopulorum* suggest an ancient incursion of that species; and (3) where the ranges of the two species meet, all recombinations of the characters of each occur in individuals of one colony. Fassett (1944a) listed two naturally occurring varieties of Rocky Mountain juniper: *Juniperus scopulorum* var. *columnaris* Fassett, a columnar form found near Amidon, North Dakota; and *J. scopulorum* var. *patens* Fassett, a depressed form found in the Big Horn Mountains of Wyoming and near Banff, Alberta.

Hall (1947) did a taxonomic study of *Juniperus* in Oklahoma and reported that eastern redcedar is generally distributed throughout Oklahoma, except in the extreme western portion which

includes the Panhandle. He concluded that Rocky Mountain juniper was not to be found in Oklahoma, although he reported specimens from northwestern Oklahoma which showed intermediate characteristics tending toward both species. His variants were similar to those described by Fassett (1944b) as hybrid swarms in the zone of overlap between the two species.

Hall (1952a) reported on a survey of *Juniperus* east of the Rocky Mountains based on studies of population samples and identified several hybrid swarms, one of which he called the "Platte River" race of eastern redcedar. Hall (1961) stated that in the Northern Great Plains there are a great many hybrids of eastern redcedar and Rocky Mountain juniper and that many horticulturally valuable variants have been found in this area by such men as D. Hill and others. He related that the parentage and percentages of germ plasm of many of these variants cannot be identified positively because the local populations from which they were obtained have not been properly studied.

Van Haverbeke (1965) made a preliminary study of the genus in Nebraska. This study verified the presence of two arborescent species: *J. virginiana* L., in the eastern two-thirds and *J. scopulorum* Sargent, in the extreme western portion of Nebraska. Interpretation of hybrid indices in this study suggested that: (1) the western population more closely resembles its pure parental type than the eastern population resembles its pure parental type; (2) specimens in the western part of the *J. virginiana* range express tendencies toward *J. scopulorum*; and (3) *J. scopulorum* may influence the juniper population throughout Nebraska, along easterly flowing river drainages, as well as in the zone of species overlap or abutment. Other taxonomic studies within the genus include those by Fassett (1943, 1945a, 1945b), Hall (1952b and 1955), Hall *et al* (1961), and Hall and Carr (1964).

Fassett (1944a) reported that *J. virginiana* and *J. scopulorum* differ in ten sets of characters:

1. percent of peduncles curved,
2. shape of leaf tips,
3. relation of gland length to distance to tip of leaf,
4. overlap of leaves,
5. width of epidermal cells,
6. branching character of stems,
7. number of years to fruit maturity,
8. size of fruit,
9. number of seeds per cone, and
10. seed markings.

Hall (1952), Hall *et al* (1961), and Hall and Carr (1964) used the following characters to distinguish various species of junipers:

1. length of terminal whip leaves,
2. gland length-width ratio,
3. distance from tip of gland to tip of leaf,
4. length of lateral whips,
5. berry-cone diameters,
6. percent of decussate spur shoots on secondary shoots,
7. leaf margins (scored as smooth, denticulate, or intermediate).

Cytological Studies

Mathews (1939) studied the morphology and cytological development of sporophylls and seed of eastern redcedar in North Carolina. He reported on the morphological and cytological development of the staminate strobilus and microsporophyll, pollen mother cells, male gametophyte, ovulate strobilus, megasporangium, female gametophyte, pollination and fertilization processes, and embryo formation.

Ross and Duncan (1949) found cytological evidences of hybridization between *J. virginiana* and *J. horizontalis* in the "Driftless Area" of Wisconsin. Their results were based primarily on a study of meiosis in pollen mother-cells of presumed hybrids and parental types. They found an imbalance in the somatic chromosome complements of suspect hybrids as evidenced by the presence of heterobrachial chromosomes without counterparts in pollen mother-cells during meiosis.

Analysis

The essence of a taxonomic analysis is the accumulation of supporting evidence from examinations of specimen characters from populations made up of variable individuals. As in most other areas of biological study, one can rarely say with absolute certainty that any one character always expresses itself in a given manner. Therefore, it is through the process of amassing evidence from various fields of investigation that one is able to establish reliable trends within or between variable populations.

Traditionally, the analyses of these data have been graphical. The methods outlined by Anderson (1949) in his study of introgression have been the basis for the majority of graphic analyses since that time. Some examples of similar and other methods of graphic treatments of taxonomic data are illustrated in the articles by Davidson (1947), Ross and Duncan (1949), Hall (1952a, 1952b), Hall *et al* (1961), and Hall and Carr (1964), Clifford (1955), Critch-

field (1957), Fowler and Heimburger (1958), Mergen (1958), Thorbjornsen (1961), and Brayshaw (1966).

With the advent of the electronic computer, a new potential has become available to taxonomists. The analysis of a population study usually involves a great number of individuals and the derivation of as many discriminating character values per individual as possible. The analysis of this multitude of data is very time consuming and is, to some extent, dependent upon the judgment and intuitive ability of the person making the analysis. For instance, in the selection of the best set of character correlations from a large number of possible combinations, it is possible for an investigator to inadvertently overlook the best pairings or groupings. Computer analysis of all possible combinations is done quickly and accurately, however. Barber (1966) described several methods of recording data for direct input into computer systems which could have application to taxonomic investigations.

Use of computers promises to: (1) save time in the analysis of the data once it is collected and thus enable the investigator to spend more time gathering data; and (2) enable new types of analyses to be developed (Sokal and Sneath, 1963; Rogers and Fleming, 1964; and Mergen and Furnival, 1960).

Materials and Methods

Field Procedures

Native stands of *Juniperus* were sampled throughout the Missouri River Basin in the fall of 1965 (Fig. 2a). Reference materials, Forest Service and University Experiment Station contacts, and the author's experience suggested that the heaviest concentrations of native juniper stands were located in, on, or near bluffs, ridges, canyons, mesas, floodplains, and uncultivated lands near and adjacent to the major rivers coursing through the Basin. Several major east-flowing rivers (Missouri, Yellowstone, Cheyenne, Niobrara, Platte, Republican, and Kansas) and their tributaries are contained within the Basin (Fig. 2a). Many of these waterways originate in the range of *Juniperus scopulorum* and flow into the range of *J. virginiana*. It was reasoned that these easterly flowing rivers could transport cones and/or seeds from the range of *J. scopulorum* into the range of *J. virginiana*. This material could establish itself for a sufficient length of time in favorable microenvironments to inject a detectable amount of *J. scopulorum* germ plasm into the *J. virginiana* population. Thus these drainage systems could serve as an excellent base from which to study the variation present within the juniper population throughout the Basin. Areas adjacent to and between the two species ranges were also considered prime areas in which variation and hybridization could be found and evaluated.

Seventy-two locations were sampled throughout the Basin. Field collections were made in two trips—a total of about 10,000 miles. The first trip in October, 1965, retraced portions of the Lewis and Clark Expedition. Juniper populations were sampled north and westward up the Missouri River from Nebraska, through the Dakotas and across Montana, and southward to the headwaters of the Missouri River; then eastward along the Yellowstone River into north and central Wyoming, northward through southeastern Montana, south through the Badlands of North and South Dakota and southwestern South Dakota, and eastward along the Niobrara River into northeast Nebraska. The second trip in November, 1965, sampled native juniper stands westward along the Platte River Valley through Nebraska and into Wyoming, southward through central

Wyoming and the eastern slope of the Rocky Mountains in Colorado, eastward through the northern half of Kansas and through Missouri to the junction of the Missouri and Mississippi Rivers; then northward through Iowa, southwestern Minnesota, southeastern South Dakota, and into eastern Nebraska.

The Missouri River Basin extends well into the natural range of *Juniperus scopulorum*; thus it was believed that this parental type would be adequately sampled within the study area. However, the Basin includes a relatively smaller portion of the natural range of *Juniperus virginiana*. In order to gain some insight as to the nature and location of the more extreme type of eastern redcedar, additional samples were collected by Forest Service and Experiment Station personnel along an east-west transect across the United States to the east coast (Fig. 2b). These samples included material from Illinois, Tennessee, Ohio, West Virginia, Virginia, North Carolina, Georgia and Connecticut.

Sampling. A minimum of ten trees, five ovulate and five staminate, old enough to bear fruiting structures and to possess mature foliage, were sampled wherever possible. At each collection site the local population was traversed on foot and inspected for variation in visible characteristics such as tree form, foliage color, sharpness of leaf-scales, size and characteristics of fruiting structures. Trees representing the extremes in visible variation were sampled first; then additional individuals lying between the extremes were sampled. The major aim of this sampling scheme was to collect enough material to insure adequate sampling of the variation present in each local population. Thus in some locations more than ten trees were sampled. However, in a few locations ten trees were not available. Sampling at a given locality was confined within an elevational bracket of about 500 feet and to an area approximately ¼ to ½ mile in diameter.

Foliage and cone collections. Four branch tips, each approximately 12 inches long or long enough to include all current years growth, possessing staminate or ovulate strobili and scale-like leaves, were cut from each sample tree. Branch tips were cut from that part of the crown fully exposed to light to avoid possible morphological differences associated with exposure. Branch tips were cut at a height of about 6 feet or at a level not exceeding one-half the height of the crown. The cut branch tips from each tree were placed in conventional botanical presses for drying and storage. Material from each sample tree was labeled with an identifying number (Example: V-M-8-10 or Van Haverbeke, Montana, collec-

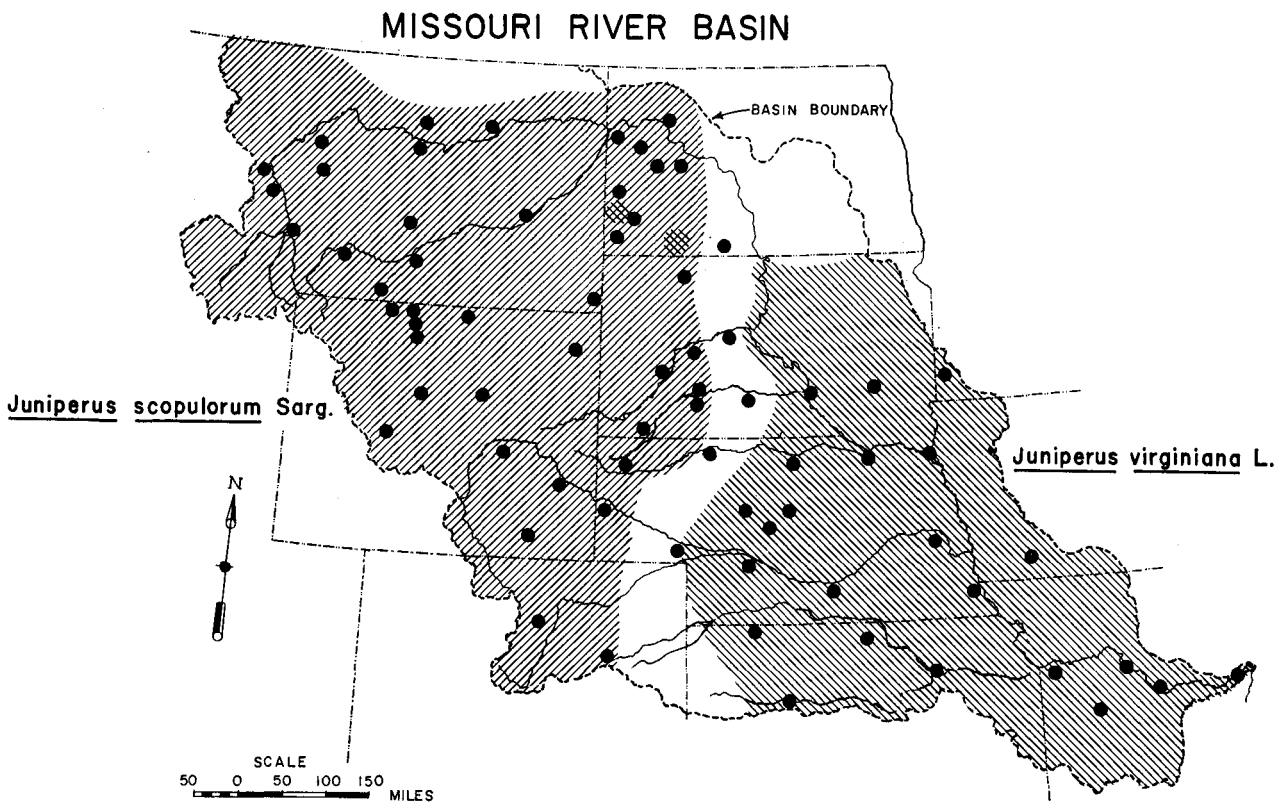


FIG. 2a.—Missouri River Basin illustrating reported species ranges of *Juniperus scopulorum* Sarg. and *J. virginiana* L., major river drainages, and locations of collection sites.

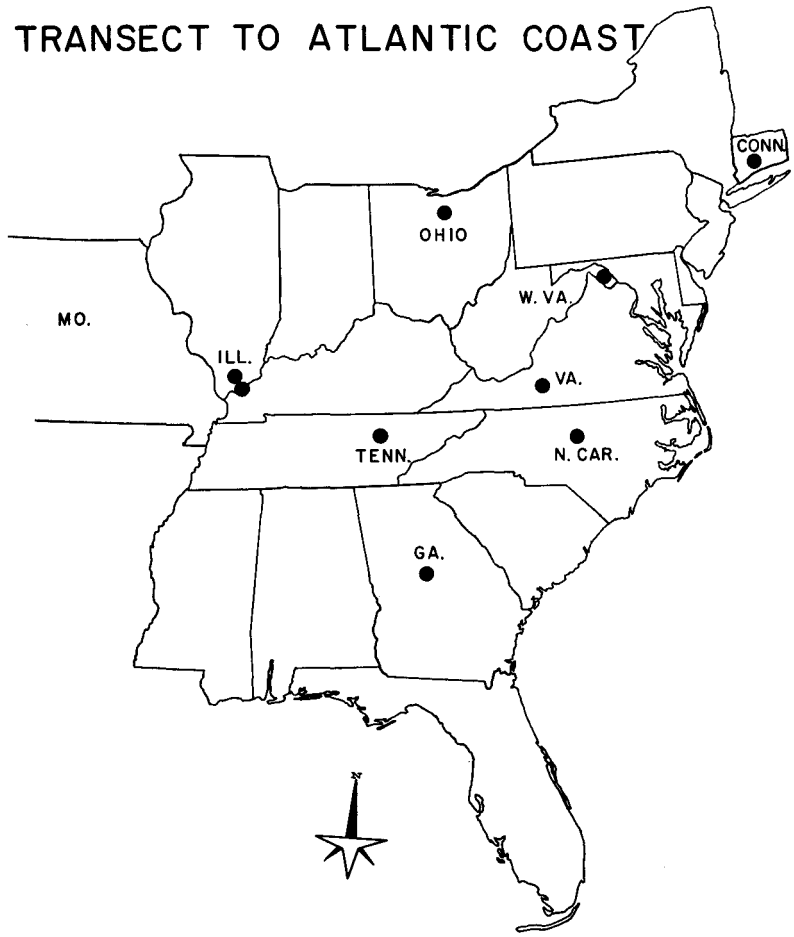


FIG. 2b.—Eastern United States showing locations of supplemental collection sites within *J. virginiana* L. range.

tion site no. 8, tree no. 10). A sample of cones was collected from each ovulate tree sampled, put in a plastic bag, labeled, and placed in cold storage as soon as possible. This material was used for determination of morphological characters of cones and seeds and of chemical data in the infrared analysis described in the Appendix.

Four branchlet segments (two tip and two side) approximately two inches long, and five staminate or five ovulate cones were also collected from each sample tree. These were placed in properly identified glass vials (two inches tall) containing a standard killing and fixing solution (FPA) as in Sass (1958). This material was col-

lected and held in reserve for possible use in future investigations or anatomical characters of foliage and staminate and ovulate reproductive structures. This material is in storage at the Lincoln Field Unit, Rocky Mountain Forest and Range Experiment Station, Lincoln, Nebraska, under study FS-RM-1501.20.

Gross morphological and topographic data. Total height, stem diameter (breast height), average crown spread, bark thickness, form habit, foliage density, foliage color, branch angle, and number of years to cone maturity were measured or evaluated for each sample tree while in the field.

Total *height* of the tree was measured in feet, and stem *diameter* was determined with a diameter tape at breast height and expressed in inches and tenths of inches. *Crown spread* was determined by measuring the width of the crown in an east-west and north-south direction to the nearest foot and averaging the two values. For *bark thickness,* an average of two measurements (to the nearest 1/20th inch at breast height) was taken with a Swedish bark gauge. *Branching habit* was determined by assigning a rating of 1 to 5 to each tree based on the number of stem-like branches (1 = single stem; 2 = forked once or twice; 3 = forked three to four times; 4 = forked five to seven times to deliquescent in form). Ratings of overall *foliage density* on a given tree were based on a numerical scale of 1 to 5 (1 = very open; 2 = open; 3 = moderately dense; 4 = dense; and 5 = very dense). *Branch angle* was determined with a protractor as the angle between the vertical axis of the tree and a representative branch ⅓ down from the top of the crown. An evaluation of *foliage color* was made by assigning a numerical value of 1 to 5 to each tree (1 = light green; 2 = green; 3 = green-blue; 4 = blue-green; and 5 = blue or glaucous-blue). *Number of years to seed maturity* was based on a visual assessment in which most eastern trees (approaching *J. virginiana*) posssessed only ripened dark-blue cones on the current season's branches; whereas most western trees (approaching *J. scopulorum*) possessed both ripened glaucous dark-blue cones on last season's foliage and unripened green cones on current season's growth.

Field observations of location, elevation, a brief topographic description, and a sketch map of each collection site were recorded. Although it was not planned to revisit the sample areas, the location of each tree was mapped; and the trees were marked by spray painting a number on the stems at breast height. This would help insure relocation of sample trees if it should be necessary to revisit them later for additional material or measurements. Photographs

were taken of representative trees and/or of general areas within certain sampling sites to help describe conditions of topography and tree form.

Laboratory Procedures

Cone and seed measurements. Cone lots of individual trees were taken out of cold storage and analyzed for the following cone and seed characters: cone length, cone width, cone length/width ratio, cone weight, cone volume, number of seeds per cone, seed length, seed width, and seed length/width ratio.

Cones were sampled for analysis as outlined in the "Mixing by Hand" method (FAO, Forest Tree Seed Directory, 1961). Cone lots from individual trees were spread out onto an open petri dish in a single layer; all damaged and deformed cones were removed; and the remaining cones were thoroughly mixed by hand. A teaspoon was dipped into the dish a minimum of five times at different areas over the petri dish to draw out a 20-cone sample. Sample size was determined for cone, seed, and foliage characters by computing and evaluating coefficients of variation for a random group of samples drawn from throughout the study area (Cochran and Cox, 1957).

Cones were measured for *length* and *width* with a Bausch and Lomb botanical dissecting microscope at a magnification of 7X (10X eyepiece x 0.7X objective lens). Cones were held and turned with a pair of hand tweezers. Determination of cone *length/width ratios* was mathematical. *Cone weight* was determined by weighing the 20 cone sample previously drawn from the cone lot as a unit on a Mettler Analytical Balance to the nearest one-thousandth gram. *Cone volume* was determined by displacing the same 20 cone sample in distilled water. A 50 ml burette tube was supported on a ringstand with two clamps and filled with water. The 20 cones were placed in a 25 ml graduate cylinder. A glass rod plunger, fitted with a rubber base, large enough to prevent the cones from floating past it to the surface, and with a wider rubber stopper positioned at a predetermined distance up the rod, was then inserted into the graduate cylinder. The rod with rubber base displaced a known amount of water. Water was then let out of the burette tube until the 25 ml graduate cylinder containing the cones was filled to a constant level. The amount of water let out of the 50 ml burette tube, minus the amount displaced by the plunger, was subtracted from the constant on the 25 ml graduate cylinder to obtain the total amount of water displaced. Cones used in the determination of cone weight and cone volume were opened for

a count of number of *seeds per cone*. Seeds were cleaned of resinous deposits by dipping them in alcohol and rubbing them dry in the palm of the hand. *Seed lengths* and *seed widths* were determined by measuring 20 seeds under the same microscope used for the cone measurements but at a magnification of 20X (10X eyepiece x 2.0X objective lens). *Seed length/width ratios* were calculated mathematically.

Crude lipid extracts from cone pulp were prepared and analyzed with a Beckman IR-10 Infrared Spectrophotometer to determine if chemical data could be obtained to strengthen and/or supplement hypotheses of species relationships based primarily on morphological data. The technique of preparation is outlined in a manuscript contained in the Appendix.

Foliage measurements. Foliage characters were measured at a magnification of 20X (10X eyepiece x 2.0X objective lens) with an American Optical Company Binocular microscope fitted with a 100 unit micro-unit scale.

Ten segments of branchlets possessing current season scale-like foliage were sampled at random from the pressed specimens. From these, five leaves were randomly selected, measured, and averaged for foliage characteristics on each specimen. *Leaf tip shapes* were qualitatively compared with a set of standard samples and assigned a ranking from 1 to 5 based on degree of sharpness (1 = acuminate; 2 = very acute; 3 = acute; 4 = sub-acute; and 5 = blunt). *Percent overlap of leaves* was determined by dividing the distance from a leaf tip to the tip of the next lower leaf by the distance from the tip of the upper leaf to the point of intersection of the lower extremities of that upper leaf with the edge of the next lower leaf, and converting to a percent value. Values were recorded as a plus, zero, or a minus percent overlap. *Leaf resin gland length* was calculated by measuring the distance from the tip of the leaf to the base of the resin gland and subtracting the distance from the tip of the leaf to the top of the resin gland. *Resin gland widths* were measured, and the *ratio of resin gland length/width* was calculated. Determinations of the *ratio of the distance from leaf tip/resin gland length* were also calculated as a foliage character.

In summary, the following characters were measured or computed for analysis:

A. *Gross morphological characters*
   1. Stem diameter
   2. Bark thickness
   3. Height

4. Crown spread
5. Ratio of height/crown spread
6. Number of years to cone maturity
7. Tree form
8. Tree density
9. Branch angle
10. Foliage color

B. *Foliage characters*

11. Leaf tip shape
12. Distance from leaf tip to leaf tip
13. Distance from leaf tip to leaf junction
14. Leaf overlap in percent
15. Distance from leaf tip to top of leaf gland
16. Distance from leaf tip to base of leaf gland
17. Leaf resin gland length
18. Ratio of leaf gland length/leaf gland width
19. Leaf resin gland width
20. Ratio of distance from leaf tip to top of leaf gland/leaf gland length

C. *Cone and seed characters*

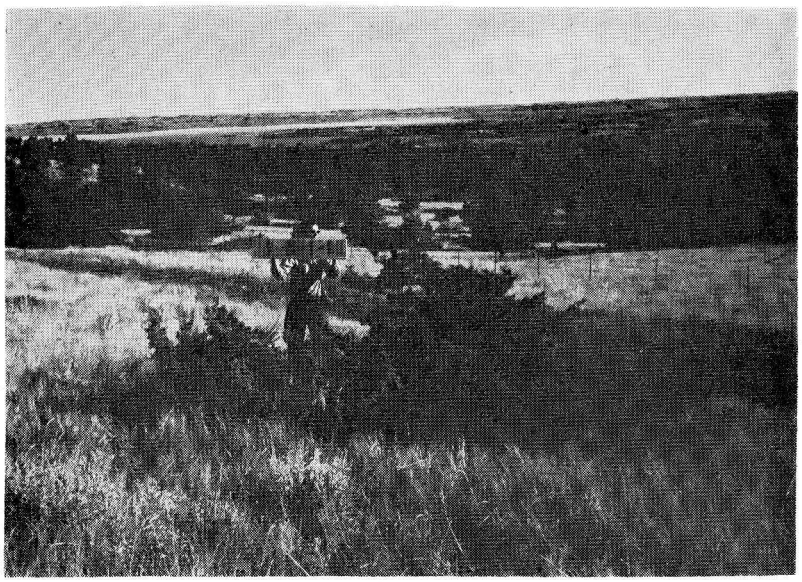
21. Cone length
22. Ratio of cone length/cone width
23. Cone width
24. Cone weight
25. Cone volume
26. Number of seeds per cone
27. Seed length
28. Ratio of seed length/seed width
29. Seed width

D. *Infrared characters* (expressed in wavenumber cm^{-1})

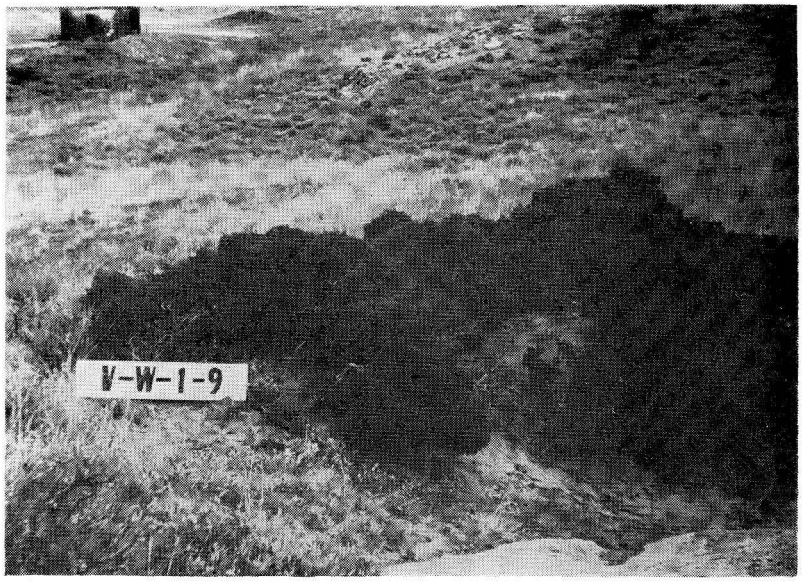
30. 2930
31. 2915
32. 2835
33. 2825
34. 1735
35. 1725
36. 1715
37. 1695
38. 1685
39. 1465
40. 1450
41. 1435
42. 1385
43. 1365

ANALYSIS

Certain specimens were encountered during the field trip in northern Wyoming, throughout much of Montana, and in the western Dakotas which suggested introgressive hybridization between *J. scopulorum* and *Juniperus horizontalis* Moench.—a prostrate spe-



(A). Mont. 1–1 (8 ft. x 20 ft.)



(B). Wyo. 1–9 (3 ft. x 10 ft.)

PLATE I.—Examples of "depressed" junipers sampled in the vicinity of: (A) Fort Peck Reservoir in Montana (Mont. 1) and (B) Elk Basin, Wyoming (Wyo. 1). Believed to be resultants from hybridization between *J. scopulorum* Sarg. and *J. horizontalis* Moench.; they are widespread throughout Montana and northern Wyoming.

cies of juniper also belonging to the *Sabina* section. These suspect hybrid individuals were at first characterized by being neither upright nor prostrate in form; rather, they were "depressed" (Plate I). Fassett (1945a) reported possible hybridization between these two junipers near Sheridan, Wyoming, west of the Big Horn Mountains in Wyoming, and near Banff, Canada. In this study, these suspected hybrid individuals were found to be widespread in their occurrence throughout the above mentioned area. Where such plants were found, members of *J. horizontalis* were usually, but not invariably, also found. A number of these depressed individuals were sampled along with the upright and apparently typical *J. scopulorum* trees. Analysis of morphological and biochemical characters of these depressed, and of certain other upright, forms from the same area, revealed a high percentage of apparently *J. horizontalis* characters mixed into the otherwise *J. scopulorum* population.

On the basis of the above, the author felt that he had passed beyond the geographical area in which *J. scopulorum-J. virginiana* data were to be found and had entered an area in which the problem concerned primarily the relationship between *J. scopulorum* and *J. horizontalis*. Since the latter problem lay outside of the scope of the present study, the suspected hybrids were screened from the study population. These data are on file under study number FS-RM-1501.20, Rocky Mountain Forest and Range Experiment Station, Lincoln, Nebraska.

One should not be so presumptuous, however, as to imply that all of the suspected *J. horizontalis* germ plasm was eliminated from the sample; but an honest attempt to do so was made without intentionally biasing the data. For instance, some evidence of this third species was detected in western North Dakota; yet some indication of *J. virginiana* influence was also detected here. Since the measured characters (except that of prostrate form vs. upright form) of *J. virginiana* and *J. horizontalis* were similar in these suspect individuals, these North Dakota specimens were retained in the analysis.

Individual tree data were transferred to standard IBM data cards for a three-stage computer analysis. First, data were processed in the "TAL" (Frequency Distribution) program in the IBM 7040 Computer.[1] This analysis involved determination of the class interval for the values derived for each variable or character. This was accomplished by determining the maximum and minimum

1. Nelson, Dale. 1966. TAL (Frequency Distribution) University of Nebraska Computing Center, June 13, 1966, mimeographed.

value for each character and dividing the range into 15 equal classes. Fifteen classes were used because of their convenience for dividing into the five classes (0 to 4) used to construct the hybrid index. The "TAL" program tabulated the frequency distributions and computed the means and standard errors for each of the 43 variables or characters derived from a total of 675 trees.

Second, the data were processed in the "CORCO" (Correlation Coefficient) program in the IBM 7040 Computer for the correlation coefficients of all possible variable (character) combinations.[2] From these data a "system" was developed involving two sets of characters which showed consistent patterns of correlation.[3] One set of characters (+, or positive) exhibited consistently positive correlations with each member of that set, but negative correlations with each member of the other set (–, or negative). The negative characters consistently showed positive correlations with each other and negative correlations with each member of the positive set. Characters which were inconsistent in the above "system" were discarded as being invalid. Of the 43 characters selected for use in the analysis, only five were eliminated from the "system." They were as follows:

A. *Gross morphological characters*
   5. Ratio of height/crown spread
B. *Foliage characters*
   13. Distance from leaf tip to leaf junction
   16. Distance from leaf tip to base of leaf gland
C. *Cone and seed characters*
   22. Ratio of cone length/cone width
   26. Number of seeds per cone

It was recognized that a few of the characters retained in the "system" were correlated with one another at levels below the usually acceptable limits of significance for simple correlations—that is, at the 1 and 5% levels. These characters, however, were retained in the "system" since their total contribution, regardless of how small, would be entirely consistent. It was also reasoned that the level of acceptance for a series of 38 characters—all showing inter-correlation—should be much lower than for a simple correlation. For instance, if one accepts the probability that in one out of twenty times character A is correlated with character B due entirely to chance, and the same odds of A also being correlated with characters C and D by chance, then this relationship of A to

2. Nelson, Dale. 1966. CORCO (Correlation Coefficients). University of Nebraska Computing Center, August 19, 1966, mimeographed.

3. Davidson, John F., Botany Department, University of Nebraska, unpublished technique.

B, C, and D could be expressed mathematically as 1/20 x 1/20 x 1/20 x 1/20 = 1/160,000; or a .00625% probability of their all being intercorrelated by chance alone. This level of probability would be infinitesimally small when carried to the n = 38th degree and should be acceptable in any analysis. The majority of the single correlations, however, were well above significance at the usual 1 and 5% levels for simple correlations.

Third, a new program called "HYBIX" (Hybrid Index) was constructed to determine a hybrid value for each variable (character) of each specimen and to compute a hybrid index value for each tree.[4] In this program, the range of values for each character retained in the "system" was divided into five equal classes (0 to 4). The computer assigned one of the above class numbers to each character value and summed them for a whole tree hybrid index value. Thus, it would be possible for a given specimen or specimens to have a hybrid index value of 0 if exhibiting a minimum hybrid value for each character—indicating one parental type. Conversely, a specimen or specimens exhibiting a maximum hybrid value of 4 for each character would constitute the other parental type. The maximum hybrid index value possible would be a value 4 times the number of characters used to evaluate the population under study.

The population sampled in this study contained both ovulate and staminate trees. Thus, the ovulate trees possessed cone and seed characteristics not present on the staminate trees. Also, it was not feasible to include all ovulate trees in the infrared analysis for chemical characteristics of cone pulp. Therefore, in order to compare all individuals with like-characters, it was necessary to analyze the data in three separate groups—that is, all ovulate and staminate trees (675 with 16 characters in common—8 gross morphological and 8 foliage); all ovulate trees (343 with 24 characters in common—9 gross morphological, 8 foliage, and 7 cone and seed); and all ovulate trees (142 with 38 characters in common—9 gross morphological, 8 foliage, 7 cone and seed, and 14 infrared). In the three groups of specimens analyzed in this study, the potential hybrid index values ranged from 0 to 64 in the 675 tree sample, 0 to 96 in the 343 tree sample, and 0 to 152 in the 142 tree sample.

4. Fuller, Marian J. 1967. The genus *Carduus* in Nebraska. Unpublished Ph.D. thesis, University of Nebraska, 92 p., illustrated.

Results and Interpretation

Frequency Distributions

Frequency distributions were constructed for data derived from 43 characters of a 675 tree sample. Characters were grouped for convenience under four categories: gross morphological (Fig. 3), foliage (Fig. 4), cone and seed (Fig. 5), and infrared (Fig. 6).

The frequency distributions for the various characters assumed a variety of forms: for example—a normal distribution (distance, leaf tip to leaf tip, Fig. 4; cone width, Fig. 5); a strongly Poisson distribution (ratio height/crown spread, Fig. 3; number of seeds per cone, Fig. 5); a strongly bimodal distribution (branch angle, Fig. 3; leaf overlap, Fig. 4; leaf resin gland length, Fig. 4); and a weakly bimodal distribution (seed length, Fig. 5; optical density at wavenumber 1735 cm^{-1}, Fig. 6).

Data relative to each character are illustrated on the appropriate figures (Fig. 3–6). These include: number of trees sampled; minimum, mean, and maximum values; and the standard deviation from the mean. Coefficients of variation ($C = \frac{s}{\bar{X}} \times 100 = \%$) were computed for all characters. These percentages ranged from a low of 9% for seed length (Fig. 5) to a high of 58% for tree form (Fig. 3). Statistical inferences are based on a normal distribution of data. Thus in this analysis, the wide range in C values was attributable more to the distributions of the character values—that is, to the shapes of the curves—than as a reflection upon the size of the sample taken or upon the reliability of the measurements.

Frequency distributions of character values are helpful in a study of plant relationships in that they are indicative of the nature of the characters and of general population relationships. For instance, when the distribution of a character shows complete bimodality—that is, the occurrence of two discrete curves on the same frequency distribution—a diagnostic character has been sampled and indicates complete separation between two distinct populations. An expression of incomplete bimodality—that is, the occurrence of two interlocking curves on the same frequency distribu-

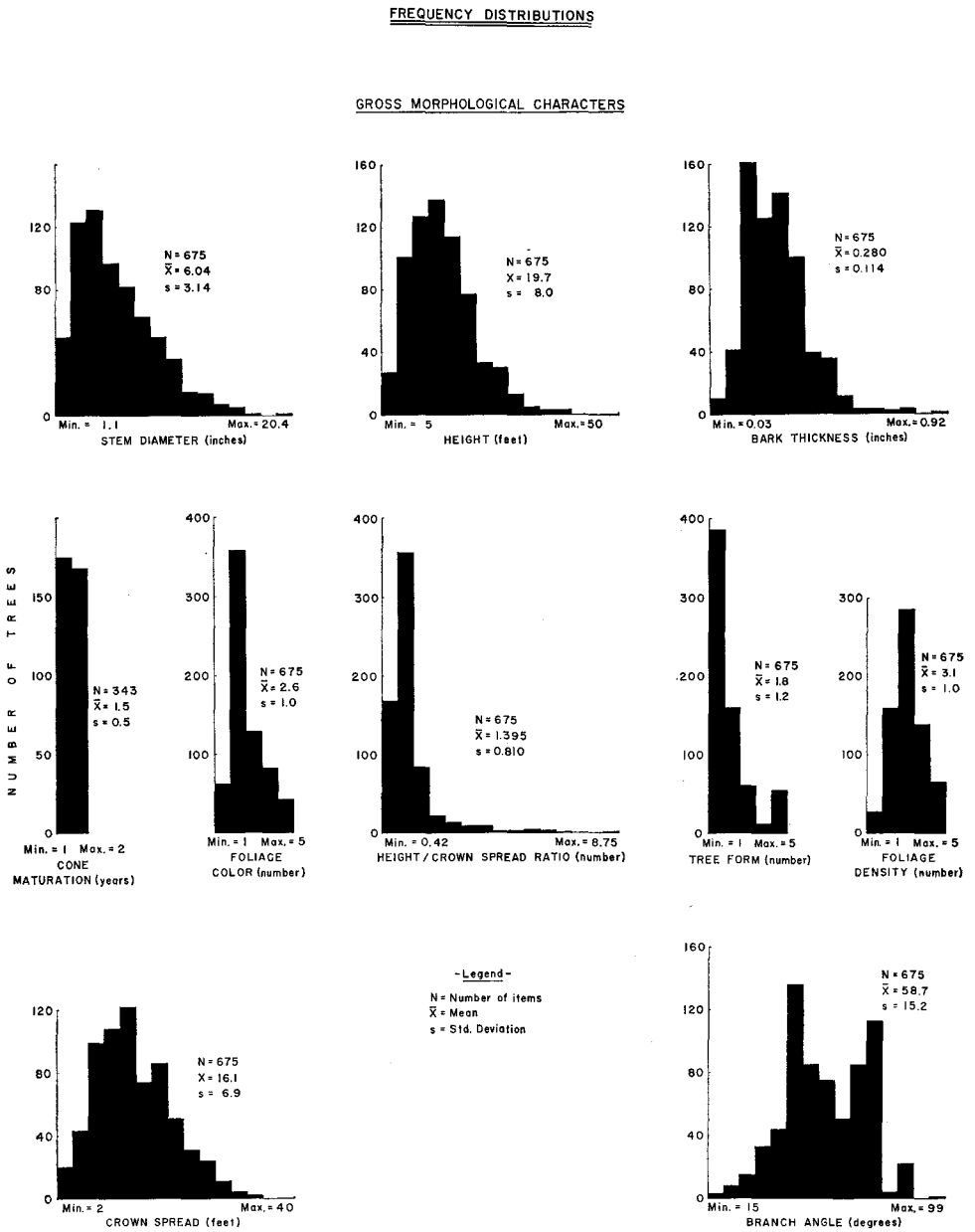


FIG. 3.—Frequency distributions of gross morphological character values.

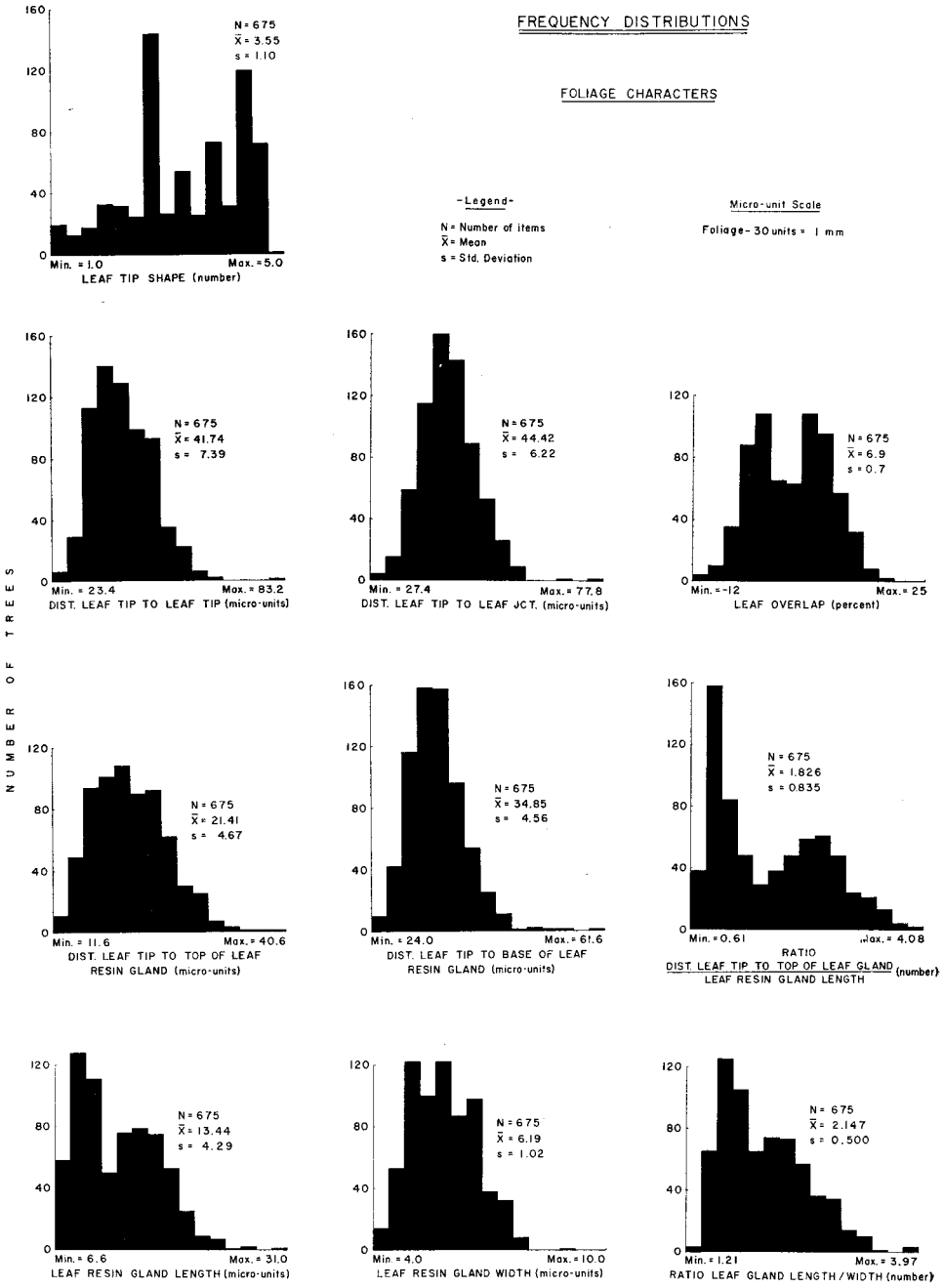


FIG. 4.—Frequency distributions of foliage character values.

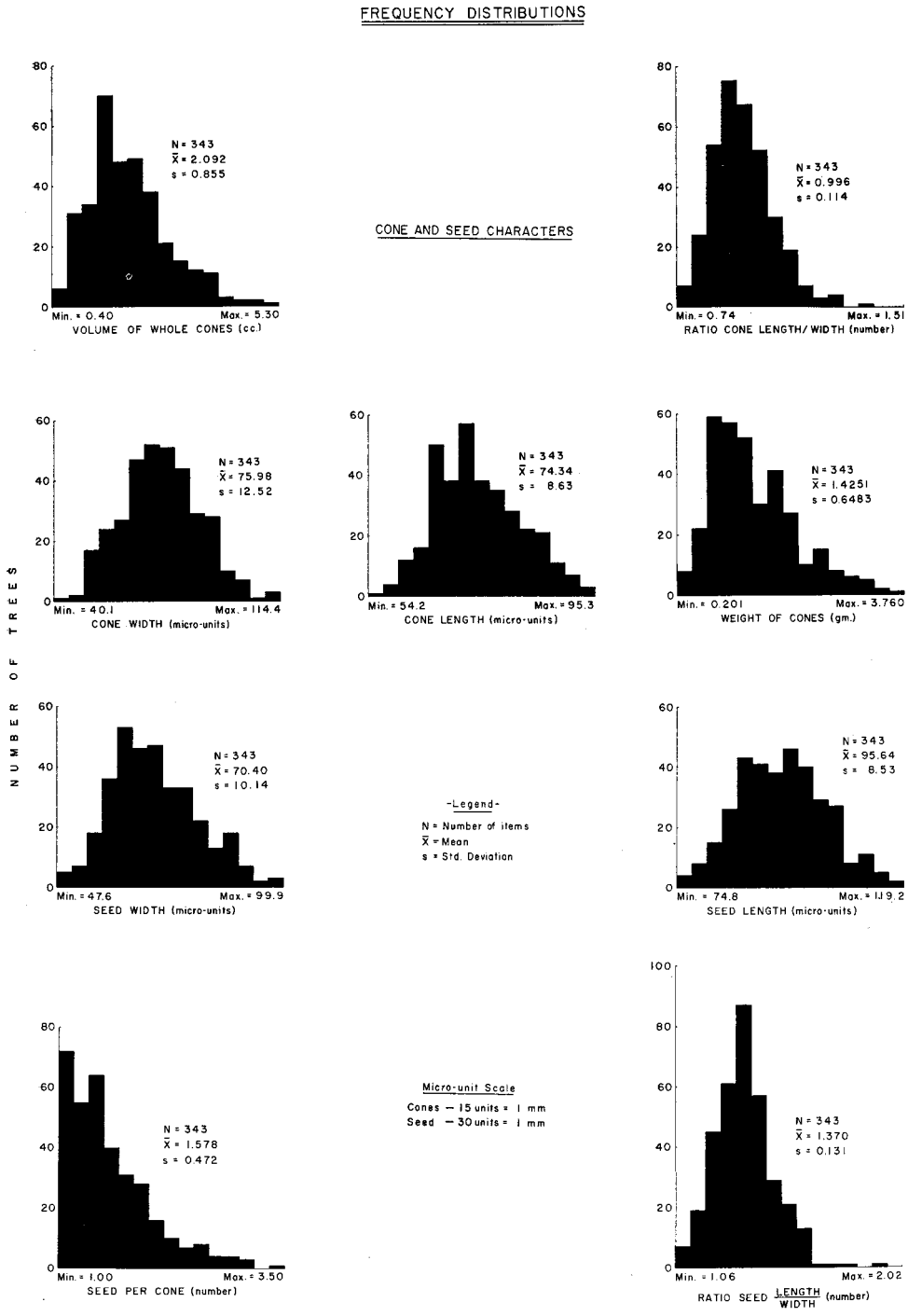


FIG. 5.—Frequency distributions of cone and seed character values.

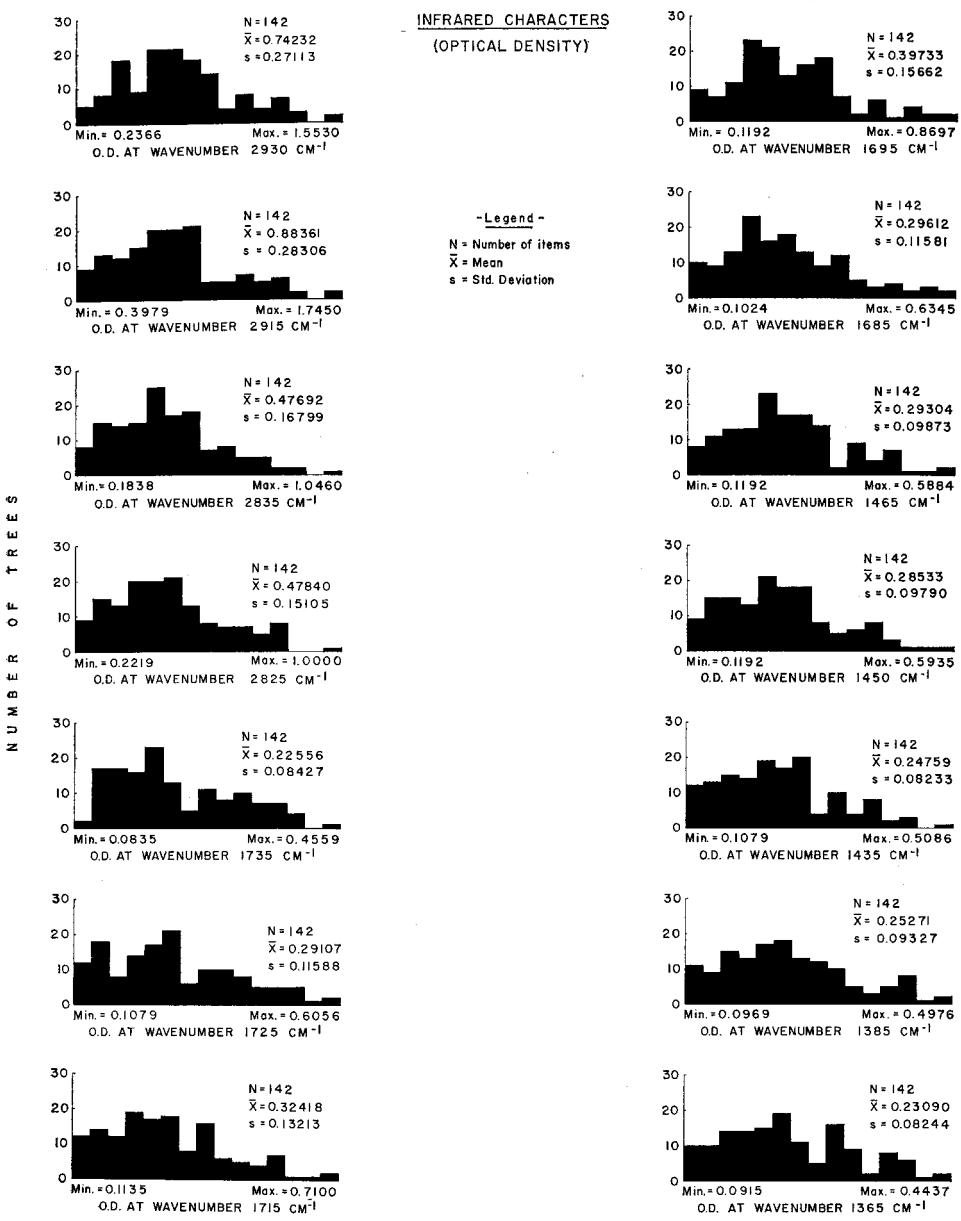


FIG. 6.—Frequency distributions of infrared character values.

tion—is suggestive of a close relationship between two different populations and reflects an intermediate condition between that indicated by two separated normal curves and the normal distribution.

Characters exhibiting strongly Poisson distributions, such as those illustrated by the characters of "ratio of height/crown spread" (Fig. 3), "tree form" (Fig. 3), and "number of seeds per cone" (Fig. 5), immediately become suspect characters. This type of distribution tends to reveal a constant, or nearly constant, value for all specimens or suggests inclusion of both young and mature data. Thus, the Poisson distribution has little or no diagnostic value.

When the values of a given character produce a normal curve when plotted, this indicates that the character variation is distributed at random throughout the population. When all characters result in a series of normal curves, it suggests that this random distribution of all characters is the result of generations of interbreeding. Whether this interbreeding population represents a single variable species or a hybrid swarm depends on whether or not correlations exist between characters. Thus the frequency distribution, *per se*, has restricted diagnostic value until the presence or absence of character correlations among this and other characters can be established. If no correlation exists among characters, the variable intrabreeding population is indicated; whereas, if correlations are established, the latter situation is suggested.

In the present study, the suggestion of bimodality in the frequency distributions of some characters suggests the presence of two germ plasms. In short, there is a slight indication, at this point, that hybridization is involved.

Correlations

Correlation coefficients (r) were established for all possible combinations among the 43 characters used to evaluate the 675 *Juniperus* tree sample (Tables 1–3). A series of significant relationships between characters was demonstrated. In order to establish these character relationships, however, it was necessary to separate the population into three sub-groups so that comparison could be made between characters common to all individuals.

The first analysis included all 675 *Juniperus* trees (ovulate and staminate) which had 9 of the 10 gross morphological characters (omitting character "years to cone maturity") and all 10 of the foliage characters in common (Table 1). The second analysis included all ovulate trees (343) with 10 gross morphological, 10 foliage, and 9 cone and seed characters (Table 2). The third analysis included 142 ovulate trees possessing the above groups of characters

Table 1.--Correlation coefficients showing relationships among gross morphological and foliage characters of 675 ovulate and staminate Juniperus trees.[1/]

	Character Number	Gross Morphological Characters										Foliage Characters									
		1	2	3	4	5 *	6	7	8	9	10	11	12	13 *	14	15	16 *	17	18	19	20
Gross Morphological Characters	1	1.000																			
	2	0.675	1.000																		
	3	0.814	0.450	1.000																	
	4	0.739	0.643	0.558	1.000																
	5*	0.007	-0.231	0.298	-0.464	1.000															
	6	-0.214	-0.163	-0.244	-0.196	-0.064	1.000														
	7	-0.236	-0.158	-0.364	0.098	-0.221	0.231	1.000													
	8	-0.259	-0.195	-0.277	-0.310	0.058	0.173	0.277	1.000												
	9	0.226	0.155	0.262	0.303	-0.036	-0.169	-0.326	-0.369	1.000											
	10	-0.063	0.041	-0.147	-0.018	-0.132	0.185	0.089	0.014	-0.047	1.000										
Foliage Characters	11	-0.378	-0.190	-0.512	-0.295	-0.231	0.273	0.320	0.183	-0.239	0.238	1.000									
	12	-0.447	-0.284	-0.501	-0.417	-0.113	0.251	0.217	0.282	-0.283	0.226	0.374	1.000								
	13*	-0.349	-0.244	-0.349	-0.331	-0.021	0.158	0.093	0.220	-0.188	0.152	0.003	0.902	1.000							
	14	0.424	0.226	0.537	0.380	0.218	-0.285	-0.326	-0.259	0.319	-0.250	-0.814	-0.683	-0.307	1.000						
	15	0.254	0.102	0.384	0.195	0.234	-0.241	-0.289	-0.106	0.222	-0.200	-0.835	-0.108	0.280	0.712	1.000					
	16*	-0.211	-0.162	-0.202	-0.190	0.005	-0.009	0.026	0.123	-0.085	0.014	-0.243	0.634	0.845	0.031	0.557	1.000				
	17	-0.495	-0.282	-0.625	-0.411	-0.243	0.248	0.336	0.245	-0.325	0.228	0.639	0.782	0.587	-0.731	-0.488	0.453	1.000			
	18	-0.411	-0.244	-0.509	-0.402	-0.135	0.249	0.317	0.269	-0.374	0.255	0.615	0.670	0.464	-0.695	-0.511	0.300	0.866	1.000		
	19	-0.408	-0.195	-0.533	-0.237	-0.320	0.137	0.217	0.108	-0.122	0.111	0.407	0.577	0.480	-0.470	-0.260	0.430	0.729	0.306	1.000	
	20	0.441	0.199	0.604	0.327	0.329	-0.277	-0.352	-0.198	0.319	-0.272	-0.813	-0.541	-0.233	0.811	0.796	-0.014	-0.869	-0.790	-0.632	1.000

0.103 = lowest r value signif. at 1% level w/674 d.f.
0.080 = lowest r value signif. at 5% level w/674 d.f.

1/ Description of characters given in Table 5.
* Characters omitted from further analysis because of failure to fit the "system".

Table 2.--Correlation coefficients showing relationships among gross morphological, foliage, and cone and seed characters of 343 ovulate Juniperus trees.[1/]

	Character Number	Gross Morphological Characters										Foliage Characters										Cone and Seed Characters								
		1	2	3	4	5 *	6	7	8	9	10	11	12	13 *	14	15	16 *	17	18	19	20	21	22 *	23	24	25	26 *	27	28	29
Gross Morphological Characters	1	1.000																												
	2	0.696	1.000																											
	3	0.819	0.513	1.000																										
	4	0.742	0.634	0.614	1.000																									
	5*	-0.049	-0.227	0.240	-0.470	1.000																								
	6	-0.505	-0.351	-0.610	-0.464	-0.191	1.000																							
	7	-0.238	-0.180	-0.390	-0.121	-0.257	0.394	1.000																						
	8	-0.290	-0.201	-0.252	-0.349	0.120	0.314	0.286	1.000																					
	9	0.209	0.138	0.285	0.316	-0.046	-0.361	-0.340	-0.369	1.000																				
	10	-0.062	0.022	-0.110	-0.056	-0.099	0.261	0.113	0.073	-0.131	1.000																			
Foliage Characters	11	-0.376	-0.259	-0.502	-0.318	-0.220	0.779	0.332	0.204	-0.288	0.304	1.000																		
	12	-0.375	-0.255	-0.458	-0.443	-0.049	0.609	0.211	0.281	-0.289	0.280	0.353	1.000																	
	13*	-0.288	-0.193	-0.321	-0.362	0.041	0.335	0.078	0.205	-0.168	0.186	-0.010	0.907	1.000																
	14	0.368	0.243	0.495	0.384	0.189	-0.805	-0.347	-0.286	0.366	-0.322	-0.814	-0.671	-0.303	1.000															
	15	0.269	0.161	0.378	0.201	0.236	-0.660	-0.300	-0.139	0.258	-0.229	-0.836	-0.075	0.303	0.707	1.000														
	16*	-0.182	-0.145	-0.201	-0.239	0.057	0.080	0.029	0.105	-0.068	0.015	-0.251	0.659	0.858	0.022	0.571	1.000													
	17	-0.488	-0.335	-0.623	-0.479	-0.185	0.791	0.350	0.265	-0.346	0.258	0.617	0.800	0.614	-0.730	-0.443	0.482	1.000												
	18	-0.405	-0.269	-0.520	-0.448	-0.093	0.743	0.331	0.328	-0.403	0.280	0.600	0.691	0.497	-0.690	-0.462	0.338	0.866	1.000											
	19	-0.408	-0.267	-0.519	-0.314	-0.252	0.534	0.229	0.082	-0.129	0.136	0.398	0.581	0.482	-0.481	-0.260	0.426	0.740	0.320	1.000										
	20	0.460	0.276	0.613	0.390	0.273	-0.839	-0.380	-0.234	0.355	-0.315	-0.815	-0.546	-0.249	0.815	0.780	-0.028	-0.863	-0.779	-0.650	1.000									
Cone and Seed Characters	21	-0.278	-0.163	-0.374	-0.198	-0.196	0.476	0.248	0.091	-0.126	0.237	0.398	0.465	0.348	-0.457	-0.289	0.192	0.515	0.383	0.483	-0.497	1.000								
	22*	0.244	0.024	0.354	0.088	0.291	-0.289	-0.129	0.046	-0.008	-0.155	-0.234	-0.210	-0.162	0.192	0.168	-0.116	-0.301	-0.212	-0.308	0.284	-0.246	1.000							
	23	-0.324	-0.118	-0.457	-0.158	-0.294	0.494	0.232	0.044	-0.086	0.254	0.392	0.440	0.335	-0.414	-0.287	0.197	0.517	0.387	0.482	-0.488	0.748	-0.798	1.000						
	24	-0.332	-0.199	-0.422	-0.214	-0.202	0.506	0.230	0.112	-0.098	0.242	0.409	0.426	0.296	-0.458	-0.324	0.139	0.493	0.377	0.449	-0.492	0.799	-0.559	0.875	1.000					
	25	-0.348	-0.162	-0.470	-0.221	-0.232	0.534	0.251	0.109	-0.102	0.234	0.425	0.486	0.364	-0.471	-0.314	0.191	0.539	0.409	0.496	-0.516	0.841	-0.612	0.940	0.933	1.000				
	26*	-0.060	0.107	-0.163	0.074	-0.230	0.026	0.074	-0.086	0.031	0.095	-0.001	0.027	0.055	0.027	0.028	0.043	0.012	-0.030	0.066	-0.001	0.149	-0.602	0.503	0.343	0.361	1.000			
	27	-0.261	-0.145	-0.338	-0.186	-0.167	0.420	0.143	0.103	-0.089	0.197	0.343	0.413	0.301	-0.425	-0.263	0.155	0.450	0.355	0.402	-0.442	0.777	-0.189	0.587	0.624	0.674	-0.024	1.000		
	28	0.109	0.121	0.105	0.122	0.003	-0.248	-0.009	0.030	0.033	-0.053	-0.231	-0.127	-0.032	0.225	0.232	0.016	-0.228	-0.160	-0.240	0.279	-0.202	0.190	-0.230	-0.316	-0.264	0.329	-0.138	1.000	
	29	-0.259	-0.189	-0.311	-0.216	-0.116	0.460	0.105	0.059	-0.096	0.174	0.390	0.371	0.226	-0.451	-0.342	0.088	0.460	0.357	0.425	-0.493	0.644	-0.243	0.537	0.626	0.618	-0.223	0.743	-0.754	1.000

0.141 = lowest r value signif. at 1% level w/342 d.f.
0.107 = lowest r value signif. at 5% level w/342 d.f.

1/ Descriptions of characters given in Table 6.
* Characters omitted from further analyses because of failure to fit the "system".

plus a series of 14 chemical characters derived from an infrared (IR) analysis of cone pulp extracts (Table 3).

The r values listed in Tables 1–3 are important in that they indicate the degree to which certain character combinations are related to one another and distinguish positive from negative correlations. Although the magnitude of some of the r values may not be impressive by non-taxonomic standards, they are, for the most part, entirely acceptable in terms of introgressive studies. A comparison of the r value and its graphic appearance can be obtained from Figures 4 and 5 of the infrared analysis contained within the Appendix. The relative strength of the character relationships is indicated in all three tables by the magnitude at which the majority of the r values exceed that required for significance between simple correlations at the commonly accepted statistical levels of 1 and 5%.

Inspection of the data reveals that the individual r values associated with a given pair of characters are well correlated in all three analyses—mainly, only the magnitude of the correlations varies. This suggests that representative samples were drawn in each of the three analyses. Correlation coefficients tend to be higher and more consistent within a given group of characters (gross morphology, foliage, cone and seed, and IR) than they are between characters belonging to different groups. For instance, foliage characters tend to be more strongly related to one another than they are to most of the gross morphological or cone and seed characters (Table 2).

Comparisons of the r values derived from intra-gross morphological character combinations and from intra-infrared character combinations (Table 3) reveal that the chemical IR characters are more strongly correlated among themselves than are the gross morphological characters correlated among themselves. The r values for the IR characters versus other group characters are also more uniform than are the gross morphological characters versus the same characters (Table 3). These conditions suggest that the IR characters may either be very closely related to one another or that the mechanical technique of deriving the IR character data is very precise, thus eliminating much of the experimental error inherent in conventional methods of character analysis. These data also suggest that gross morphological characters, such as height, crown spread, etc., may be less reliable for use as taxonomic characters, since they are external characters of long duration. Consequently, they are subject to more severe and drastic alteration from atypical

or unusual environmental phenomena, such as livestock damage, snow, ice, hail, and sand blasting. Internal chemical characters are probably less subject to the ravages of the environment.

The correlations presented in Tables 1–3 were the basis for selecting the characters used in the "system" described in the Materials and Methods section. It can be readily seen in Table 3 that those characters (*) which failed to meet the qualifications of the "system" were those characters which generally exhibited the lowest correlation coefficient values. This method was less restrictive than a purely statistical approach to character selection in that it accepted not only those characters that would have been included on the basis of significance at a given level but also all of those which were consistently correlated, regardless of the size of the r value. The major criteria for acceptance was that they all be consistently correlated either above or below zero correlation within the appropriate group of characters (positive or negative). Table 4 lists the characters which were selected for the "system" and those which were rejected.

Data contained within Tables 1–3 enable one to interpret the frequency distributions (Figs. 3–6) more accurately which, until the relationships among individual characters were established by correlations, were of restricted diagnostic value. For instance, the suspect nature of characters whose frequency distribution values were arrayed in a strongly Poisson distribution can now be confirmed, as evidenced by the rejected characters of 5 (ratio of height/crown spread) and 26 (number of seeds per cone) from the "system." These characters also exhibited low r values—having character values with such restricted ranges that it was not possible to establish any relationships or trends between them and any other characters. In short, they possessed a nearly constant value for all the trees within the sample. Characters 13 (distance from leaf tip to leaf junction), 16 (distance from leaf tip to base of leaf resin gland), and 22 (ratio of cone length/cone width), whose distributions are normal, are apparently characters whose values are so randomly distributed throughout the population sampled that no significant trends of relationship could be established.

The essence of a taxonomic evaluation of any given population of organisms is the establishment of the presence of groups of characters which are associated (correlated) within segments of the population under study—thus indicating a genetic relationship.

Ownbey and Aase (1955, as in Benson, 1962, p. 276) stated: "The chances of a large series of correlated resemblances coming about

Table 3.--Correlation coefficients showing relationships among gross morphological foliage, cone and seed, and infrared chemical characters of 142 ovulate Juniperus trees.[1/]

Character Number		1	2	3	4	5 *	6	7	8	9	10	11	12	13 *	14	15	16 *	17	18	19	20	21	22 *	23	24	25	26 *	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
Gross Morphological Characters	1	1.000																																									
	2	0.709	1.000																																								
	3	0.819	0.557	1.000																																							
	4	0.788	0.596	0.722	1.000																																						
	5*	-0.121	-0.130	0.160	-0.457	1.000																																					
	6	-0.445	-0.301	-0.528	-0.504	0.002	1.000																																				
	7	-0.277	-0.178	-0.394	-0.165	-0.238	0.333	1.000																																			
	8	-0.275	-0.159	-0.244	-0.345	0.255	0.294	0.244	1.000																																		
	9	0.397	0.222	0.429	0.492	-0.223	-0.432	-0.361	-0.463	1.000																																	
	10	-0.032	-0.001	-0.082	-0.062	0.012	0.214	0.217	0.142	-0.211	1.000																																
Foliage Characters	11	-0.331	-0.209	-0.438	-0.355	-0.066	0.776	0.331	0.217	-0.360	0.272	1.000																															
	12	-0.362	-0.266	-0.416	-0.525	0.234	0.592	0.209	0.315	-0.329	0.283	0.317	1.000																														
	13*	-0.228	-0.153	-0.234	-0.373	0.261	0.243	0.097	0.216	-0.156	0.179	-0.132	0.873	1.000																													
	14	0.395	0.306	0.492	0.499	-0.064	-0.815	-0.279	-0.304	0.434	-0.295	-0.830	-0.659	-0.215	1.000																												
	15	0.291	0.198	0.393	0.306	0.110	-0.713	-0.236	-0.179	0.387	-0.182	-0.880	-0.144	0.321	0.784	1.000																												
	16*	-0.113	-0.071	-0.120	-0.201	0.165	-0.082	0.114	0.126	-0.022	-0.020	-0.428	0.513	0.800	0.204	0.643	1.000																										
	17	-0.490	-0.328	-0.628	-0.601	0.035	0.811	0.421	0.360	-0.511	0.208	0.651	0.713	0.451	-0.770	-0.573	0.259	1.000																								
	18	-0.400	-0.264	-0.529	-0.514	0.047	0.792	0.423	0.398	-0.533	0.245	0.640	0.619	0.335	-0.725	-0.596	0.111	0.870	1.000																							
	19	-0.390	-0.251	-0.469	-0.436	0.003	0.420	0.180	0.130	-0.216	0.063	0.336	0.518	0.390	-0.445	-0.237	0.348	0.671	0.229	1.000																						
	20	0.448	0.285	0.594	0.523	0.038	-0.830	-0.370	-0.308	0.504	-0.248	-0.831	-0.511	-0.116	0.853	0.829	0.143	-0.892	-0.810	-0.579	1.000																					
Cone and Seed Characters	21	-0.312	-0.157	-0.366	-0.302	-0.045	0.502	0.263	0.063	-0.245	0.289	0.435	0.492	0.315	-0.503	-0.356	0.075	0.530	0.416	0.419	-0.503	1.000																					
	22*	0.333	0.086	0.409	0.229	0.158	-0.355	-0.142	-0.020	-0.054	-0.156	-0.213	-0.285	-0.236	0.189	0.127	-0.135	-0.305	-0.229	-0.238	0.211	-0.291	1.000																			
	23	-0.376	-0.155	-0.449	-0.347	-0.076	0.526	0.220	0.056	-0.150	0.316	0.392	0.508	0.369	-0.436	-0.298	0.144	0.531	0.415	0.404	-0.448	0.793	-0.779	1.000																		
	24	-0.393	-0.268	-0.458	-0.407	-0.007	0.602	0.217	0.130	-0.280	0.332	0.479	0.517	0.323	-0.536	-0.405	0.088	0.606	0.494	0.452	-0.561	0.884	-0.524	0.873	1.000																	
	25	-0.396	-0.206	-0.465	-0.403	-0.000	0.578	0.239	0.120	-0.237	0.314	0.445	0.569	0.399	-0.516	-0.351	0.136	0.589	0.472	0.438	-0.515	0.863	-0.611	0.948	0.923	1.000																
	26*	-0.104	-0.042	-0.147	-0.064	-0.102	0.003	0.052	-0.102	0.149	0.082	-0.062	0.008	0.082	0.121	0.093	0.062	-0.052	-0.099	0.007	0.128	0.249	-0.552	0.493	0.350	0.382	1.000																
	27	-0.346	-0.169	-0.405	-0.289	-0.072	0.456	0.203	0.071	-0.260	0.220	0.390	0.458	0.282	-0.488	-0.338	0.065	0.498	0.417	0.362	-0.486	0.771	-0.280	0.643	0.674	0.698	-0.005	1.000														
	28	0.144	0.103	0.201	0.279	-0.112	-0.423	0.011	-0.096	0.186	-0.109	-0.348	-0.298	-0.097	0.442	0.354	0.030	-0.414	-0.342	-0.336	0.473	-0.175	0.037	-0.143	-0.300	-0.212	0.347	-0.138	1.000												
	29	-0.334	-0.201	-0.407	-0.383	0.026	0.576	0.128	0.108	-0.301	0.217	0.476	0.503	0.254	-0.614	-0.457	0.021	0.599	0.499	0.456	-0.627	0.617	-0.216	0.520	0.650	0.602	-0.210	0.746	-0.756	1.000											
Infrared Characters	30	-0.293	-0.133	-0.404	-0.306	-0.054	0.560	0.281	0.136	-0.345	0.324	0.547	0.357	0.108	-0.542	-0.524	-0.091	0.563	0.506	0.375	-0.633	0.511	-0.255	0.464	0.429	0.463	0.017	0.427	-0.283	0.461	1.000										
	31	-0.279	-0.147	-0.395	-0.298	-0.054	0.568	0.283	0.143	-0.352	0.353	0.555	0.336	0.082	-0.546	-0.536	-0.113	0.556	0.509	0.359	-0.640	0.486	-0.221	0.426	0.407	0.431	0.002	0.404	-0.301	0.456	0.990	1.000									
	32	-0.294	-0.142	-0.408	-0.311	-0.053	0.566	0.273	0.132	-0.344	0.308	0.547	0.352	0.103	-0.539	-0.524	-0.089	0.566	0.506	0.379	-0.634	0.485	-0.255	0.446	0.416	0.445	0.018	0.417	-0.289	0.458	0.994	0.991	1.000								
	33	-0.282	-0.152	-0.393	-0.306	-0.042	0.569	0.271	0.141	-0.344	0.332	0.549	0.339	0.086	-0.542	-0.534	-0.108	0.558	0.515	0.354	-0.639	0.460	-0.223	0.413	0.387	0.414	0.001	0.395	-0.287	0.441	0.985	0.994	0.991	1.000							
	34	-0.312	-0.194	-0.431	-0.357	-0.059	0.773	0.313	0.245	-0.401	0.404	0.687	0.451	0.128	-0.703	-0.665	-0.152	0.675	0.680	0.335	-0.756	0.551	-0.252	0.476	0.489	0.504	-0.032	0.449	-0.343	0.509	0.844	0.867	0.847	0.865	1.000						
	35	-0.311	-0.156	-0.436	-0.347	-0.054	0.677	0.318	0.176	-0.387	0.331	0.617	0.427	0.143	-0.628	-0.596	-0.095	0.649	0.604	0.391	-0.707	0.524	-0.272	0.487	0.461	0.499	0.014	0.447	-0.300	0.482	0.968	0.968	0.971	0.970	0.926	1.000					
	36	-0.284	-0.131	-0.413	-0.322	-0.057	0.630	0.292	0.155	-0.380	0.312	0.581	0.399	0.132	-0.590	-0.564	-0.093	0.611	0.566	0.373	-0.670	0.501	-0.266	0.468	0.437	0.476	0.016	0.434	-0.283	0.463	0.974	0.971	0.978	0.975	0.896	0.991	1.000				
	37	-0.250	-0.099	-0.375	-0.306	-0.043	0.550	0.209	0.157	-0.340	0.236	0.511	0.368	0.129	-0.530	-0.506	-0.087	0.545	0.482	0.365	-0.592	0.421	-0.277	0.420	0.387	0.413	0.023	0.402	-0.246	0.419	0.880	0.871	0.894	0.892	0.786	0.901	0.930	1.000			
	38	-0.264	-0.126	-0.388	-0.329	-0.020	0.560	0.204	0.121	-0.340	0.238	0.513	0.379	0.134	-0.541	-0.519	-0.088	0.561	0.484	0.392	-0.611	0.425	-0.266	0.422	0.399	0.416	0.010	0.422	-0.292	0.466	0.888	0.879	0.902	0.899	0.784	0.903	0.928	0.982	1.000		
	39	-0.270	-0.092	-0.388	-0.288	-0.058	0.561	0.269	0.148	-0.356	0.308	0.541	0.340	0.097	-0.526	-0.522	-0.101	0.550	0.505	0.352	-0.623	0.484	-0.235	0.431	0.395	0.435	0.019	0.401	-0.262	0.426	0.984	0.981	0.986	0.981	0.850	0.793	0.979	0.896	0.893	1.000	
	40	-0.281	-0.116	-0.401	-0.299	-0.060	0.575	0.279	0.144	-0.358	0.315	0.554	0.349	0.101	-0.537	-0.533	-0.096	0.569	0.520	0.366	-0.642	0.494	-0.236	0.436	0.411	0.441	0.023	0.412	-0.274	0.442	0.984	0.984	0.990	0.985	0.860	0.978	0.983	0.896	0.898	0.995	1.000	
	41	-0.296	-0.144	-0.420	-0.307	-0.076	0.583	0.275	0.127	-0.343	0.305	0.566	0.338	0.086	-0.537	-0.543	-0.105	0.572	0.512	0.387	-0.650	0.507	-0.254	0.455	0.440	0.459	0.040	0.429	-0.285	0.460	0.978	0.977	0.986	0.976	0.849	0.965	0.970	0.891	0.896	0.984	0.992	1.000	
	42	-0.319	-0.138	-0.444	-0.334	-0.067	0.617	0.316	0.170	-0.382	0.318	0.598	0.380	0.111	-0.588	-0.567	-0.099	0.609	0.556	0.392	-0.681	0.533	-0.242	0.463	0.453	0.479	0.025	0.444	-0.278	0.465	0.981	0.975	0.981	0.971	0.880	0.978	0.975	0.871	0.871	0.987	0.989	0.982	1.000
	43	-0.342	-0.169	-0.471	-0.358	-0.069	0.650	0.327	0.173	-0.384	0.325	0.623	0.389	0.109	-0.608	-0.592	-0.106	0.632	0.582	0.399	-0.706	0.535	-0.258	0.475	0.464	0.489	0.023	0.477	-0.294	0.477	0.972	0.967	0.972	0.963	0.900	0.978	0.970	0.855	0.859	0.974	0.980	0.997	0.993	1.000

0.215 = lowest r value signif. at 1% level w/141 d.f.
0.164 = lowest r value signif. at 5% level w/141 d.f.

1/ * Descriptions of characters given in Table 7.
* * Characters omitted from further analysis because of failure to fit the "system".

hybrid values in the following analyses will hereafter be identified with the parental type of *J. virginiana*; and the maximal potential hybrid values will be identified with the parental type of *J. scopulorum* (Fig. 7a).

The basic data illustrated in Figure 7a show potential hybrid values of 0 for *J. virginiana* and 64 for *J. scopulorum*. However, actual values of only 14 and 57 were calculated for trees approaching *J. virginiana* and *J. scopulorum*, respectively. These data suggest that the entire population was of a hybrid derivation, since neither extreme parental type was found. Figure 7a suggests, however, that there are two recognizable germ plasm systems present within the Missouri River Basin—witness the distinct tendency toward bimodality. These data further suggest that trees in the *J. scopulorum* portion of the distribution possessed a slightly higher percentage of *J. scopulorum* germ plasm than did the trees in the *J. virginiana* side of the distribution possess *J. virginiana* germ plasm (Fig. 7a). This was indicated by differences of 7 and 14 units between actual and potential hybrid values for the two species, respectively. These data also suggest that there is interchange of germ plasm between the two populations—witness the area of overlap in the distribution range in the general area of 30 to 40 (Fig. 7a). The basic data illustrated in Figure 7a are grouped in Figure 7b. These data afford a clearer view of the nature of this sample based on a large number of individuals with relatively few characters.

Table 5.—Gross morphological and foliage characters used to analyze 675 ovulate and staminate *Juniperus* trees throughout the Missouri River Basin and across a transect to the Atlantic Coast.

Char. No.	Gross Morphological	Char. No.	Foliage
1	Stem diameter	11	Leaf tip shape
2	Bark thickness	12	Dist. from leaf tip to leaf tip
3	Height	14	Leaf overlap in percent
4	Crown spread	15	Dist. from leaf tip to top of leaf gland
7	Tree form	17	Leaf resin gland length
8	Foliage density	18	Ratio leaf gland length/leaf gland width
9	Branch angle	19	Leaf resin gland width
10	Foliage color	20	Ratio of dist. from leaf tip to top of leaf gland/leaf gland length
	Total 16 characters		

This analysis further suggests that, within the limited samples collected to the east beyond the boundaries of the Missouri River Basin, the extreme parental type of *J. virginiana* was also not sampled (Figs. 7a, 7b, and Table 8). It is likely that these eastern specimens did not contain pure *J. virginiana* germ plasm but were

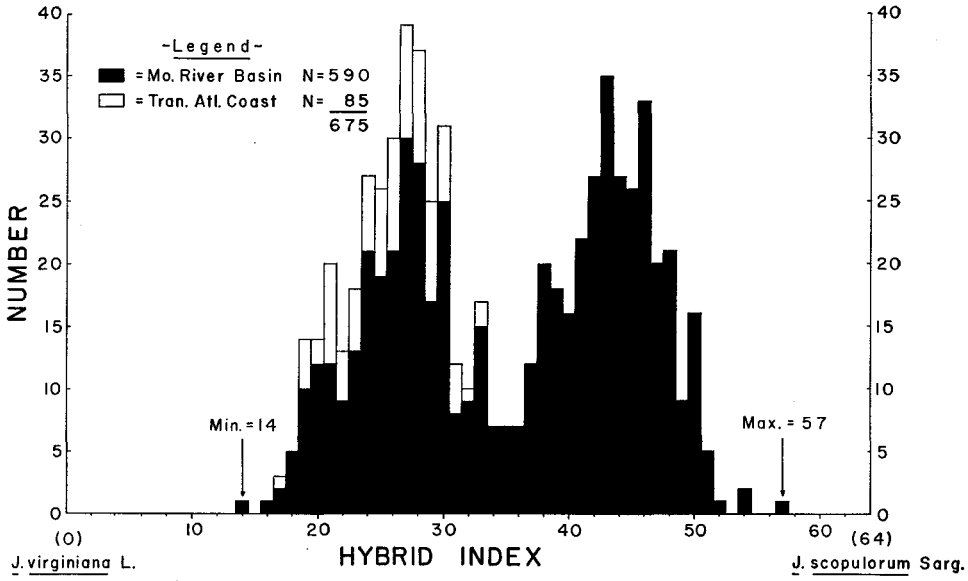


FIG. 7a.—Frequency distribution (16 characters) (basic data).

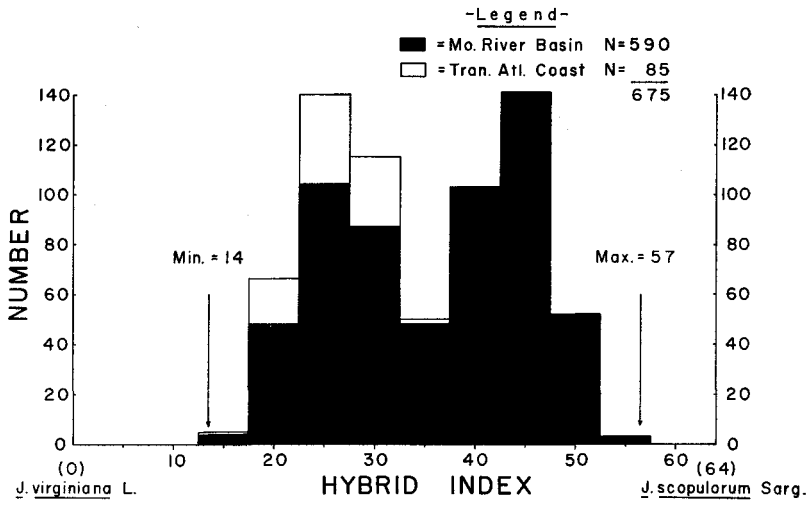


FIG. 7b.—Frequency distribution (16 characters) (basic data grouped).

segments of this or other introgressive complexes, as reported by Hall (1952a). Data in Table 8 indicate, however, that from among the eastern samples, the Tennessee sample most nearly approached the extreme of the *J. virginiana* parental type.

If most juniper populations reflect some degree of introgressive hybridization among two or more species, then it would indeed be a difficult task to find an individual or individuals which expressed either the minimum or maximum hybrid value for every character being evaluated. This probability becomes even more remote when one considers that these "pure" individuals are located at the extremes of the normal distribution; and, therefore, their numbers would be much fewer than for those individuals possessing any other intermediate genetic constitution between the parental types. This, coupled with the fantastically low survival ratio for most seed bearing plants (probably about one in one million), would further reduce the chances of sampling a "pure" individual.

The geographic array of the computed hybrid values, based on the mid-point of the range of values at a given site, reveals a trend of increasing values from southeast to northwest over the study area—that is, from the reported range of *J. virginiana* to and into the range of *J. scopulorum* (Fig. 8). These data reveal an increase in hybrid values from the lower 20's in Missouri and southeastern Nebraska; into the mid-20's in central Nebraska and Kansas; to the high 20's in western Nebraska, western Kansas, and the eastern half of South Dakota. Then a discernible break in the data is evident along the western edge of a zone of hybrid values comparable to an F_1 type beginning in extreme western Kansas, extending northward into western Nebraska, diagonally northeastward through southwestern South Dakota and the Badlands, then northward into central North Dakota. Here, these values break rather sharply upwards from the high 20's and about 30 to the middle and high 30's. In this analysis based on a maximum potential hybrid value of 64, the most intermediate type—the F_1—should be expected to exhibit a potential hybrid value of 32. However, since the entire population apparently reflects a relatively strong *J. scopulorum* influence, this F_1 value could logically be increased to about 35 (½ of the 7 unit difference between the potential versus actual hybrid values). Figures 7a and 7b reveal that values from 30 to 40 calculated for trees sampled at sites adjacent to and along this F_1 zone are, in fact, those values which lie within the area of overlap between the two population distributions and, therefore, indicate intermediacy.

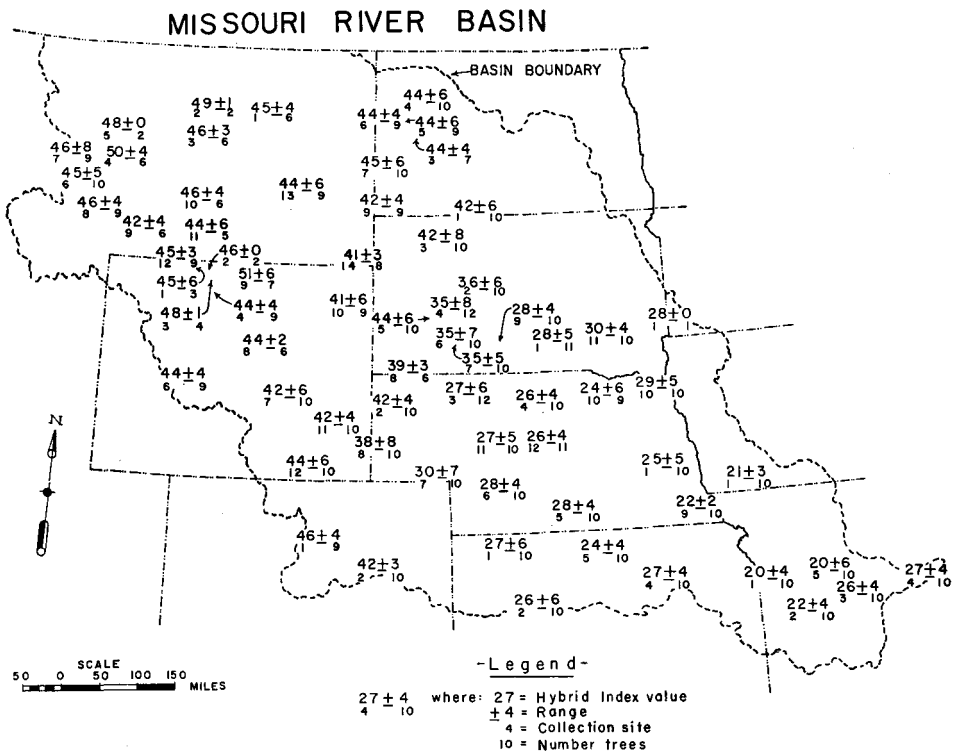


FIG. 8.—Geographic array of hybrid index values derived from 590 *Juniperus* trees and utilizing 16 gross morphological and foliage characters (see also Table 8).

These data suggest that, based on the sample analyzed, one could logically separate the study population into two major groups —those with hybrid values of, say, 30 or less would be tending toward *J. virginiana* and those with hybrid values of, say, 40 or greater would be tending toward *J. scopulorum*. Those with hybrid values of about 31 to 39 would represent trees with highly variable character combinations from both parental types. North and west of the F_1 zone the hybrid values gradually increase into the middle and high 40's to about 50 as one proceeds through central Colorado, Wyoming, North Dakota, and Montana.

In introgressive studies the tendency for variation to decrease as the parental types are approached is expected. With reference to Figure 8, it can be seen that the greatest variation (±6 and above) from the mid-value in the hybrid index generally occurs along the zone of intermediacy (F_1); and the variation generally decreases (±6 and below) as the location of the parental types is approached.

Some higher hybrid indices than might be expected were recorded in samples from sites in eastern South Dakota (HI:30), eastern Nebraska (HI:29), Missouri (HI:26 and 27) (Fig. 8). These higher values support the hypothesis that there may have been a movement or extension of *J. scopulorum* germ plasm via floating cones and seeds down the Missouri River drainage from the Dakotas. The larger tributaries of the upper Missouri River may have offered a more extensive collecting and transporting system for juniper cones and seeds than did the tributaries of smaller drainages associated with such rivers as the Platte and others. There was visible evidence of this influence in the gross appearance of occasional trees as far east as Jefferson City, Missouri (Site #3). The role of birds, such as cedar waxwings, jays, juncos, grosbeaks, crossbills, and others, in distributing seeds is significant over short distances.

The data also suggest an influence of *J. virginiana* germ plasm into the *J. scopulorum* range along the Platte River Valley from Nebraska into Wyoming. This is reflected in the slowly ascending cline of hybrid values proceeding westward along the Platte River (Fig. 8). There is also variation in the hybrid values throughout Wyoming and Montana (Fig. 8).

As outlined in the Materials and Methods section under Analysis, evidence was found in northern Wyoming and throughout much of Montana suggesting the influence of a third species, *J. horizontalis* Moench. (Plate I). The measured characters for this species, with the exception of tree form, were more similar to those of *J. virginiana* than to those of *J. scopulorum*. If the *J. horizontalis-scopulorum* complex represents an introgressive population of junipers—and this seems likely—then it is improbable that all of this influence was completely screened from the specimens retained in the analysis. Thus, some individuals would tend to exhibit lower actual values than otherwise "pure" *J. scopulorum*. Specific locations where these suspect introgressants (*J. scopulorum* Sarg. var. *patens* Fassett) were observed and sampled were numerous—Montana 1, 2, 3, 5, 7, 9, 11, 12 and Wyoming 1, 2, 3, 8, and 9; see Fig. 8 for site locations. The increased variability at Montana 6 (±5) and also at Montana 7 (±8) suggests intrusion by other germ plasm. In support of this, material identifiable as *J. horizontalis* was found in the general area.

In addition to introgression of *J. horizontalis* into the *J. scopulorum* population, additional unexplained variation was found. Several factors may be responsible. The area is vast; thus the environmental factors are much varied with respect to topography,

soil types, precipitation, water availability, temperature extremes, etc. The influence of either a very broad, non-selective environment or the presence of a great number of restricted but highly selective environments could result in the widespread genetic variability found within this region. The latter seems more likely in view of the isolated and spotty distribution of *J. scopulorum* as observed in the field and suggested in Figure 1. This, in turn, could lead to the formation and preservation of ecotypic variations due to the separation of habitats. Unusual or isolated environmental regimes might therefore be expected to select out and perpetuate unique combinations of characters. The Yellowstone River drainage, for example, may contain a unique environment as compared with more severe environs outside its influence. It is hypothesized that the noticeably different juniper population along the Yellowstone River in western and central Montana may have originated by means of one of the above processes. For the most part these junipers appear to be much denser (almost as if sheared) "flame-shaped," and greener in color than the usual *J. scopulorum* (Montana 6, 7, 8, 9, 12, and to a degree also at Montana 10, 11, and 13; see Fig. 8 for site locations).

Also, a population of columnar junipers was observed near a burning coal vein near Amidon, North Dakota. This population of extreme variants was given varietal status by Fassett (1945c) as *J. scopulorum* Sarg. var. *columnaris* Fassett. Similar groups or individuals, however, were observed and sampled at Wyoming 9, near Sheridan, Wyoming; at Colorado 2, near Golden, Colorado; at Nebraska 12, near Taylor, Nebraska; and at Missouri 4, west of St. Louis, Missouri (see Fig. 8 for site locations).

Twenty-four gross morphological, foliage, and cone and seed characters were used in the second analysis of 343 ovulate *Juniperus* trees (Table 6). These data also demonstrated a strong tendency toward bimodality supporting the interpretations from the first analysis (Figs. 9a and 9b). Again, neither potential hybrid value (0 or 96) was found. A minimum actual value of 22 and maximum actual value of 81 were recorded. The maximum actual value was 7 units closer to the maximum potential than was the minimum actual value to the minimum potential value. This analysis again suggests that, in general, this hybrid population contains a relatively higher percentage of *J. scopulorum* germ plasm than it does *J. virginiana* germ plasm. Figures 9a and 9b again confirm the presence of two not completely pure germ plasm systems within the area sampled and indicates an area of germ plasm exchange (hybrid

values of 40–50). The potential F_1 value would be about 48, which corresponds closely with Figures 9a and 9b.

Table 6.—Gross morphological, foliage, and cone and seed characters used to analyze 343 ovulate *Juniperus* trees throughout the Missouri River Basin and across a transect to the Atlantic Coast.

Char. No.	Gross Morphological	Char. No.	Foliage
1	Stem diameter	11	Leaf tip shape
2	Bark thickness	12	Dist. from leaf tip to leaf tip
3	Height	14	Leaf overlap in percent
4	Crown spread	15	Dist. from leaf tip to top of leaf gland
6	Years to cone maturity		
7	Tree form	17	Leaf resin gland length
8	Foliage density	18	Ratio leaf gland length/leaf gland width
9	Branch angle		
10	Foliage color	19	Leaf resin gland width
		20	Ratio dist. from leaf tip to top of leaf gland/leaf gland length

Char. No.	Cone and Seed
21	Cone length
23	Cone width
24	Cone weight
25	Cone volume
27	Seed length
28	Ratio seed length/seed width
29	Seed width
	Total 24 characters

Samples outside the Basin (Figs. 9a, 9b, and Table 8) revealed a similar trend as in the first analysis; that is, the extreme parental type of *J. virginiana* was not sampled. However, the hybrid values

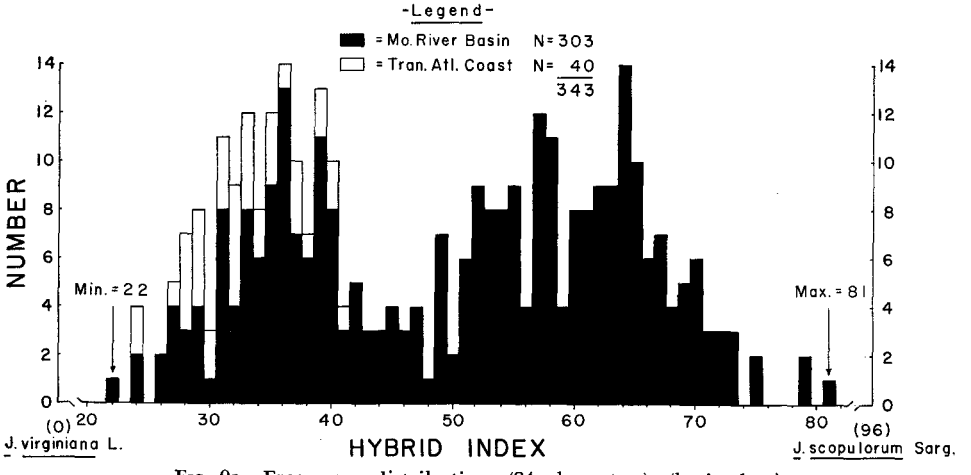


FIG. 9a.—Frequency distribution (24 characters) (basic data).

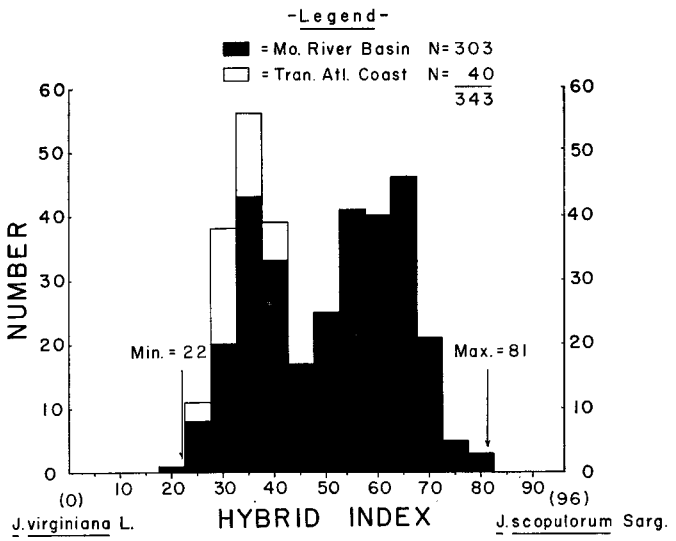


FIG. 9b.—Frequency distribution (24 characters) (basic data grouped).

of these trees tended to array themselves toward the minimal side of the *J. virginiana* portion of the hybrid index frequency distribution (Figs. 9a and 9b).

The geographic array of hybrid values illustrated in Figure 10 was similar to that of the first analysis; that is, there was an apparent pattern of increasing hybrid values from southeast to northwest throughout the study area with a more discernible break in the values along the western edge of the southwest-northeast F_1 zone described in the previous analysis. The hybrid values shown in Figures 9a and 9b correspond closely with the locations of hybrid values indicated in Figures 7a and 7b. Proceeding westward into central Nebraska and central South Dakota, one can detect a general pattern of increasing hybrid values within the *J. virginiana* population. Then the hybrid values corresponding to the region of overlap in Figures 9a and 9b are found within and along the southwest-northeast F_1 zone described above. To the west are found increasing hybrid values which are associated with those trees approaching *J. scopulorum* (Fig. 10). This transition from one population to the other can be followed up the Platte River Valley where changes in environmental conditions would tend to be more gradual than on upland sites. A relatively high percentage of *J. virginiana* germ plasm is reflected in the hybrid values of trees sampled in northeast Wyoming (site 10) and southwest Montana site 14). Values of 56 are on the low side of the *J. scopulorum*

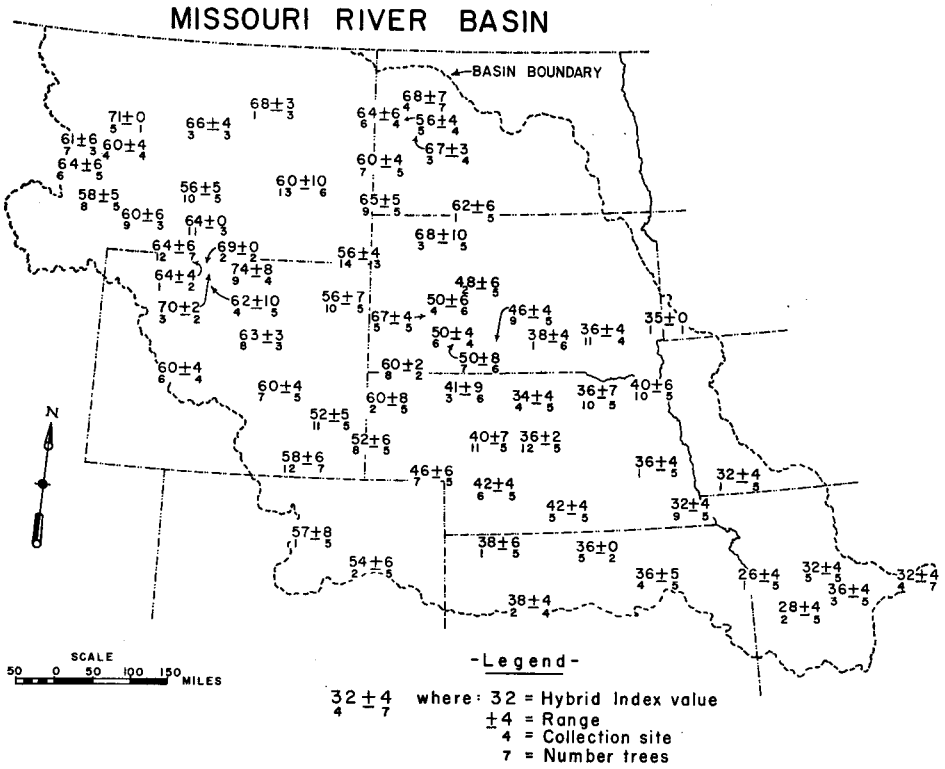


FIG. 10.—Geographic array of hybrid index values derived from 303 *Juniperus* trees and utilizing 24 gross morphological, foliage, and cone and seed characters. (See also Table 8.)

curve in Figures 9a and 9b. An influence of *J. virginiana* germ plasm in the *J. scopulorum* population was also observed and measured in southwestern and northwestern North Dakota (sites 7 and 6). The range (±) values seem to increase conspicuously within the F_1 zone. To the west of this zone these values are less meaningful, however, because of the reduced numbers of individuals sampled at a given location (Fig. 10).

The third analysis involved a total of 38 gross morphological, foliage, cone and seed, and infrared characters determined from 142 trees (Table 7). Hybrid indices derived from these data again demonstrate a strong tendency toward bimodality (Figs. 11a and 11b). This analysis, which contained a limited number of trees but a larger number of characters, contrasted with the first analysis in which a large number of trees with fewer characters was used. (Tables 5 and 7).

In this analysis the range of potential hybrid values separating

parental types was from 0 to 152 (Figs. 11a and 11b). Again neither extreme parental value was found, and the population was nearly equidistant between the parental types with a slight tendency toward *J. virginiana* (6 units closer). With a potential range of 152 hybrid units, this is probably not significant, however.

The geographic array of hybrid values derived from this analysis showed a similar, but perhaps not as consistent, pattern as was demonstrated in the two previous analyses (Fig. 12). Reduced numbers of trees sampled, fewer sampling sites, increased numbers of characters, and the precision of the infrared technique could have accounted for these variations of the general pattern. The first two reasons would tend to reduce the uniformity of the data; whereas, the last two would tend to increase the precision of the data obtained.

Table 7.—Gross morphological, foliage, cone and seed, and infrared chemical characters used to analyze 142 ovulate *Juniperus* trees throughout the Missouri River Basin and across a transect to the Atlantic Coast.

Char. No.	Gross Morphological	Char. No.	Foliage
1	Stem diameter	11	Leaf tip shape
2	Bark thickness	12	Dist. from leaf tip to leaf tip
3	Height	14	Leaf overlap in percent
4	Crown spread	15	Dist. from leaf tip to top of leaf gland
6	Years to cone maturity	17	Leaf resin gland length
7	Tree form	18	Ratio leaf gland length/leaf gland width
8	Foliage density	19	Leaf resin gland width
9	Branch angle	20	Ratio dist. from leaf tip to top of leaf gland/leaf gland length
10	Foliage color		

Char. No.	Cone and Seed	Char. No.	Infrared (wavenumber cm^{-1})
21	Cone length	30	2930
23	Cone width	31	2915
24	Cone weight	32	2835
25	Cone volume	33	2825
27	Seed length	34	1735
28	Ratio seed length/seed width	35	1725
		36	1715
29	Seed width	37	1695
		38	1685
		39	1465
		40	1450
		41	1435
		42	1385
		43	1365
	Total 38 characters		

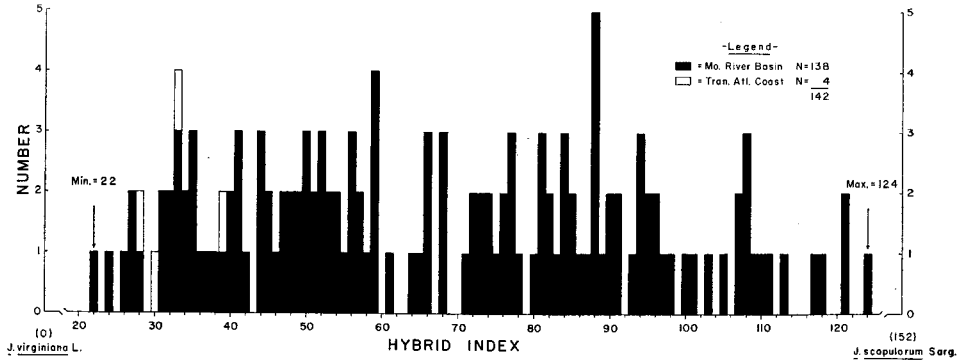


FIG. 11a.—Frequency distribution (38 characters) (basic data).

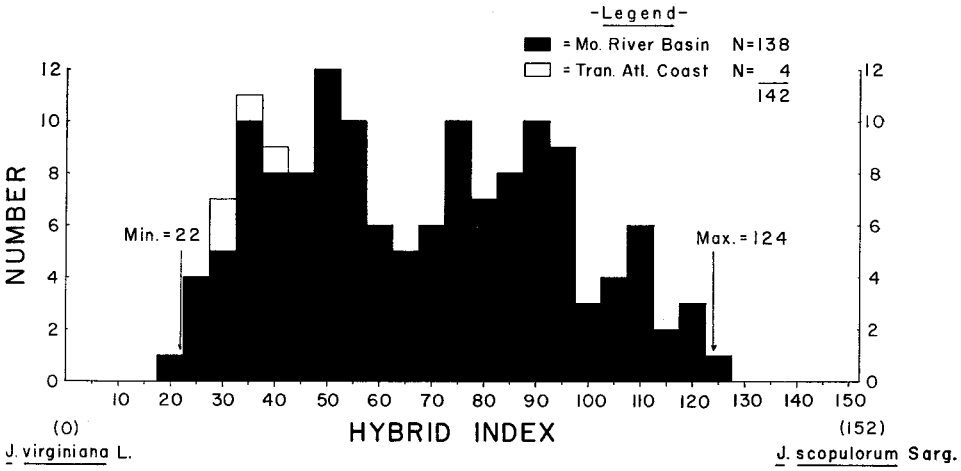


FIG. 11b.—Frequency distribution (38 characters) (basic data grouped).

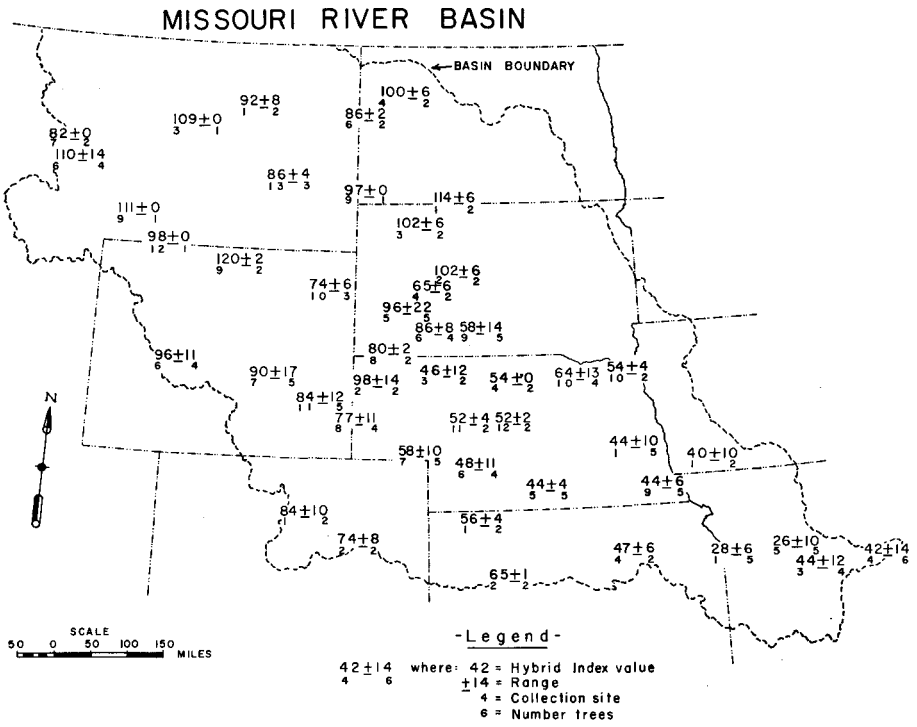


FIG. 12.—Geographic array of hybrid index values derived from 138 *Juniperus* trees and utilizing 38 gross morphological, foliage, cone and seed, and infrared characters. (See also Table 8.)

Hybrid values increase in a northwest direction with values contained within the *J. virginiana* segment of the hybrid indices extending westward to the F_1 zone between the two populations as in the previous analyses (Figs. 11b and 12). The westward extension of the eastern germ plasm along the Platte River Valley is very prominent and diagnostic (44, 48, 58, 84, 90, and 96 in western Wyoming). The potential F_1 value would be about 76 or slightly less (Figs. 11a and 11b). The magnitude between actual hybrid values 58 and 78 is indicative of the sharp increase in values on the western side of this F_1 zone. This same trend can be observed in western South Dakota where hybrid values change even more abruptly from 58 to about 90 with a hybrid value of 65 located at Cedar Pass, South Dakota (site 6) where the *Juniperus* population has long been thought to be of hybrid derivation (Fig. 12). The abruptness of the transition between the two populations of juniper is more pronounced in this analysis than in either of the two pre-

Table 8.—Hybrid index values determined for *Juniperus* trees sampled eastward to the Atlantic Coast from the Missouri River Basin (includes collection sites, number of trees, range mid-point ± limits of range).

State	Site No.	No. Trees	Hybrid Index Data Range mid-point ± limits
(A) Included in 675 tree, 16 character analysis			
Connecticut	1	10	26.0 ± 4.0
No. Carolina	1	10	26.0 ± 5.0
Virginia	1	5	24.5 ± 2.5
Georgia	1	10	26.5 ± 5.5
W. Virginia	1	10	26.0 ± 5.0
Ohio	1	10	25.0 ± 5.0
Tennessee	1	10	21.5 ± 2.5
Illinois	1	10	25.0 ± 4.0
	2	10	27.0 ± 6.0
(B) Included in 343 tree, 24 character analysis			
Connecticut	1	5	33.0 ± 2.0
No. Carolina	1	5	30.5 ± 6.5
Georgia	1	5	30.5 ± 3.5
W. Virginia	1	5	35.5 ± 5.5
Ohio	1	5	37.5 ± 2.5
Tennessee	1	5	28.0 ± 4.0
Illinois	1	5	32.0 ± 3.0
	2	5	33.5 ± 5.5
(C) Included in 142 tree, 38 character analysis[1]			
Georgia	1	2	30.5 ± 2.5
W. Virginia	1	1	30.0 ± 0.0
Tennessee	1	1	39.0 ± 0.0

[1] These data must be viewed with some caution because of the limited sample size.

vious ones. Hybrid values within the range of 55 to 75 are common throughout this zone—fitting nicely the numerical range of overlap in Figure 11b. This situation is to be expected, since this F_1 zone closely parallels the region of rapid topographic change from a gradual rise in elevation across the Plains to a more abrupt rise into the Black Hills and the Rocky Mountains. Hence the total of all the environmental conditions—not elevation alone—becomes more rigorous in its selection of genotypes tending toward *J. scopulorum*. The elevation again decreases in northeast Wyoming, southeast Montana, and western North Dakota. Here a different set of environmental conditions—more similar in their total effects to those found in the western extremities of the *J. virginiana* range—tend to select out genotypes for survival tending toward *J. virginiana* (Wyo. 10, Mont. 14, and No. Dak. 6 and 7).

The variation in the range values (±) of the hybrid values should, perhaps, be viewed with some caution in this analysis due to the small and varying numbers of trees sampled at a given site (Fig. 12). However, the variability in hybrid values is generally

higher in and near the F_1 zone between the two populations as would be expected in a hybridization series.

Figure 12 also tends to support the hypothesis stated earlier pertaining to an extension of western type germ plasm into the more easterly type via the Missouri River. Also, the extension of *J. virginiana* germ plasm into eastern Wyoming and the variation throughout Montana are still apparent. Likewise, the influence of *J. scopulorum* is evident in the western portion of the population included in the *J. virginiana* distribution, since hybrid values included in the *J. scopulorum* portion of the frequency distribution of Figure 12 are located geographically east of the F_1 zone and within the area formerly identified as *J. virginiana.*

There is a changing pattern of environmental conditions from southeast to northwest throughout the Missouri River Basin which closely parallels the changing pattern of hybrid values. Annual precipitation decreases, elevation and latitude increase, annual minimum temperatures decrease, and climatic types change from moist-humid to semiarid (Fowells, 1965). Since members of two species differ in their genetic constitution, this difference is reflected in their biochemical, physiological, and morphological characters. In the junipers studied, it is also reflected in their environmental tolerances. Environments in which the various factors are of an intermediate intensity would tend to select for survival those trees which are intermediate in genetic constitution. Hence it should be expected that there would be a range of intermediate genetic constitutions viable only in the corresponding ranges of intermediate environmental conditions. The trees would thus exhibit a corresponding range of intermediate biochemical, physiological, and morphological characters.

Some slightly lower hybrid values than those recorded in Tennessee were calculated in Missouri (Table 8, Figs. 8, 10, and 12). Admittedly these eastern collections represent an inadequate sample relative to the size and diversity of the area covered; and they were intended to serve only as a supplement to the main collection. The macro- and, certainly, the micro-environmental conditions represented within this sample were undoubtedly quite variable as suggested by a range in elevation from 350 feet in Georgia to 2,100 feet in Virginia. In dealing with two genetically dissimilar species such as *J. virginiana* and *J. scopulorum,* one should probably expect also that the greatest dissimilarities in environments would select the most dissimilar germ plasms. Thus one might logically expect to find the most typical *J. virginiana* somewhere along or near the

lower Mississippi River Drainage near the southern limit of its range. Hall (1952a) found the best development of the Tennessee habit form of the race *typica* of *J. virginiana* on the Lebanon limestone of the Nashville Basin. Our sample from Tennessee was east of this but still within the area outlined by Hall to contain junipers most nearly approaching *J. virginiana*. The other collections, with the exception of the Illinois samples, were outside or on the periphery of this area.

Data in Figure 12 and in Figure 2 of the Appendix reveal a situation which is not so apparent in the previous 675 and 343 tree analyses based entirely on morphological data. The hybrid values (Fig. 12) and the optical density values (Fig. 2 in Appendix) calculated for trees on rather arid, rocky, less fertile, upland sites in western Kansas, central Nebraska, and central South Dakota are higher than for sites further west along the fertile Platte and Niobrara River Valleys. The micro-environments in these upland areas tend to be more like those environments commonly found in more westerly locations. Consequently, the hybrid indices—as influenced by optical density—found in these areas are more in the magnitude of those found in the west or in the *J. scopulorum* environs. Since the environment is quite rigorous in its selection for the genotype that is to survive and since the genotype determines the chemical constitution of the plant, it may well be that the infrared technique provides a more precise means of discriminating between micro-environments than can be obtained by more conventional methods. Additional investigation of this aspect of variation with infrared might further strengthen the diagnostic potential of the infrared technique developed in this study.

The next logical step in this analysis might be to re-evaluate maps portraying the species ranges of the two major plant populations and their sub-divisions in accordance with the data obtained. This, however, would tend to weaken the stated objectives of the study—that of determining the occurrence and extent of the variation within the study area. Although this investigation has confirmed the existence of two basically different patterns of junipers within this area of hybridity and has indicated an area where the species might be separated, it has also revealed a situation that would invariably be masked by the erection of such arbitrarily established specific boundary lines—that is, the existence of directional trends strongly indicating interchange of germ plasm between the two species. Definite delineations of taxon ranges tend to cause one to interpret all the plants contained within such boundaries in

terms of homogeneity—that is, all black or all white. Thus in the junipers studied, one tends to think of all junipers within the confines of the *J. scopulorum* range as being entirely *J. scopulorum* and all junipers within the *J. virginiana* range as being all *J. virginiana* (Fig. 2a). In reality, this is not the case, since these data indicate the presence of some germ plasm from both species throughout the entire population. Most biological populations—as do the junipers evaluated in this study—vary from some shade of very dark gray to some shade of very light gray. It would, therefore, be difficult if not impossible to construct accurately a series of line drawings on a map to denote degrees of hybridity within this population. There would always be the exceptional plant or plants which would occur on the "wrong" side of the boundary line. Therefore, it may be more accurate and informative to let the data presented in Figures 8, 10, and 12 represent the situation as it exists in nature.

This would accurately emphasize the following concept: it matters little what we call the juniper population or populations within the Missouri River Basin as long as we recognize that variability exists within this, as well as any other, natural population. To sub-divide this population by erecting arbitrary boundaries around segments of it would mask this concept and tend to cause us to again view the junipers within these restricted boundaries as being, to a large degree, homogeneous. This concept of variation within the juniper population is illustrated in the following series of photographs which depicts the gradual transition in gross morphology of *Juniperus* from east to west and emphasizes some of the variation prevalent in the *J. scopulorum* portion of the range (Plate II).

Hybrid Index to Percent Hybridity

The hybrid index analyses were fundamental to the evaluation of this *Juniperus* population; and, to the taxonomist, these data are sufficiently diagnostic to provide strong evidence of species interrelationships. However, it is appreciated that the interpretation of such data may not be immediately apparent to some other botanists; it may be more important to such individuals to simply know how the junipers vary within a given area.

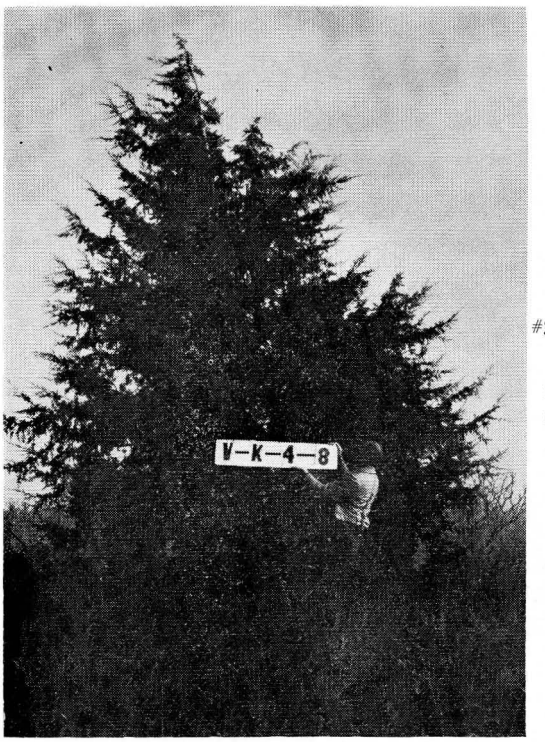
There was a striking similarity in the results of the three analyses involving varying numbers of trees and characters. The second analysis, consisting of 343 ovulate trees with 24 gross morphological, foliage, and cone and seed characters, was chosen for the following

PLATE II.—Transition in gross morphological appearance of *Juniperus* trees across the Missouri River Basin from the range of *J. virginiana* (east) to and into the range of *J. scopulorum* (west); also emphasizing the variation found within the *J. scopulorum* population throughout its range. (Numbers are keyed to a picture on following pages).

#1 *Missouri—Site 4*

#2 *South Dakota—Site 10*

LEFT: Junipers with dense, columnar, excurrent form, approaching *J. virginiana*. RIGHT: Junipers with excurrent, more open-growth habit, gently ascending branches with extended branch tips, and a generally equilateral triangular form found throughout western portion of *J. virginiana* range within the Missouri River Basin.



#3 Kansas—Site 4

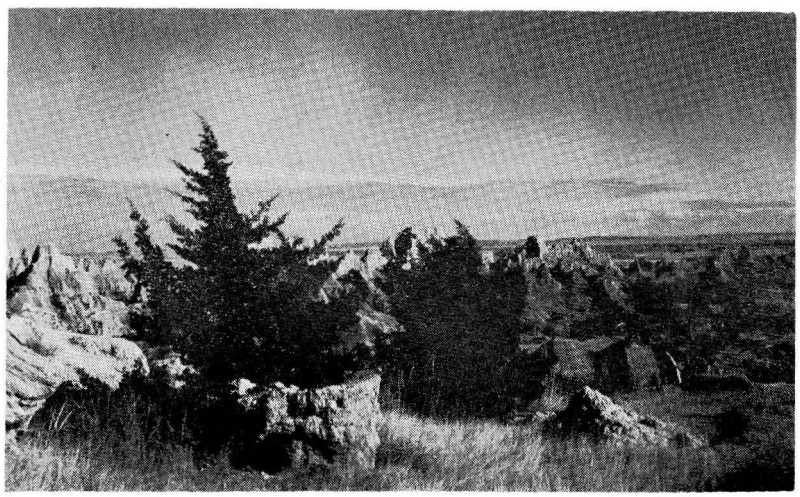
Same general conformation as in #2 with slight variations.

#4 Nebraska—Site 11

Junipers approaching the western extremity of *J. virginiana* range. Showing tendencies toward deliquescence, bushier habit, larger cones, and other characters tending toward *J. scopulorum*.

#5 Nebraska—Site 7

Area of evident hybridity. Excurrent forms tending toward *J. virginiana* (left) and deliquescent forms tending toward *J. scopulorum* (right) are prevalent. All combinations of parental characters are common; however, tendency toward *J. virginiana* is slightly stronger.

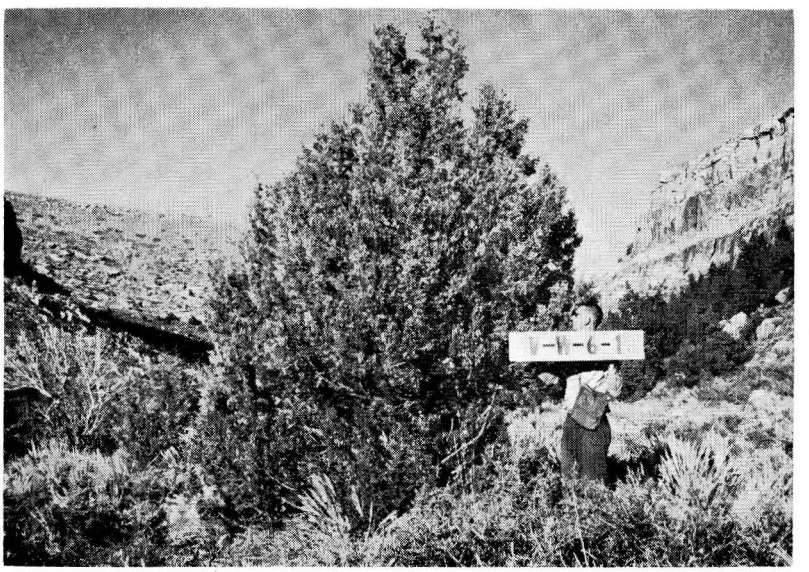


#6 South Dakota—Site 6

Similar conditions exist here as described in #5 above, except that tendency toward *J. scopulorum* is slightly stronger.

#7 South Dakota—Site 5

J. scopulorum prevalent in area only a few miles west of site #6 in South Dakota above. *J. scopulorum* characters becoming more obvious.



#8 Wyoming—Site 6

Predominantly *J. scopulorum.* (In nature blue color of foliage is notable.)

#9 *Montana—Site 13*
Predominantly *J. scopulorum.*

#10 *Montana—Site 7*
Illustrates wide variation in *Juniperus* within the reported *J. scopulorum* range.

Table 9.—Table for determination of hybrid index.

No.	Characters[1] Name	Hybrid Values 0	1	2	3	4
	Gross Morphological					
1	Stem diameter	21.0–17.1	17.0–13.1	13.0–9.1	9.0–5.1	5.0–1.1
2	Bark thickness	.97–.79	.78–6.0	.59–.41	.40–.22	.21–.03
3	Height	56–46	45–35	34–24	23–13	12–2
4	Crown spread	46–38	37–29	28–20	19–11	10–2
6	Years to cone maturity	–	1	–	2	–
7	Tree form	1	2	3	4	5
8	Foliage density	1	2	3	4	5
9	Branch angle	94–87	86–69	68–51	50–33	32–15
10	Foliage color	1	2	3	4	5
	Foliage					
11	Leaf tip shape	.08–1.7	1.8–2.7	2.8–3.7	3.8–4.7	4.8–5.7
12	Distance leaf tip to leaf tip	23.4–35.4	35.5–47.5	47.6–59.6	59.7–71.7	71.8–83.8
14	Leaf overlap (%)	+30–+22	+21–+13	+12–+4	+3—5	—4—12
15	Distance leaf tip to top leaf gland	41.3–35.4	35.3–29.4	29.3–23.4	23.3–17.4	17.3–11.4
17	Leaf resin gland length	6.6–11.5	11.6–16.5	16.6–21.5	21.6–26.5	26.6–31.5
18	Ratio leaf gland length/leaf gland width	1.21–1.77	1.78–2.34	2.35–2.91	2.92–3.48	3.49–4.05
19	Leaf resin gland width	3.8–5.1	5.2–6.5	6.6–7.9	8.0–9.3	9.4–10.7
20	Ratio distance leaf tip to top leaf gland/leaf gland length	4.15–3.45	3.44–2.74	2.73–2.03	2.02–1.32	1.31–0.61
	Cone and Seed					
21	Cone length	54.2–62.5	62.6–70.9	71.0–79.3	79.4–87.7	87.8–96.1
23	Cone width	40.1–55.0	55.1–70.0	70.1–85.0	85.1–100.0	100.1–115.0
24	Cone weight	.201–.913	.914–1.626	1.627–2.339	2.340–3.052	3.053–3.765
25	Cone volume	.38–1.37	1.38–2.37	2.38–3.37	3.38–4.37	4.38–5.37
27	Seed length	74.8–83.7	83.8–92.7	92.8–101.7	101.8–110.7	110.7–119.7
28	Ratio seed length/seed width	2.10–1.90	1.89–1.69	1.68–1.48	1.47–1.27	1.26–1.06
29	Seed width	47.6–58.1	58.2–68.7	68.8–79.3	79.4–89.9	90.0–100.5
	Hybrid Index =	0 (*J. virginiana*)		48 (F_1)		96 (*J. scopulorum*)

[1] Methods of character measurement are described in Materials and Methods.

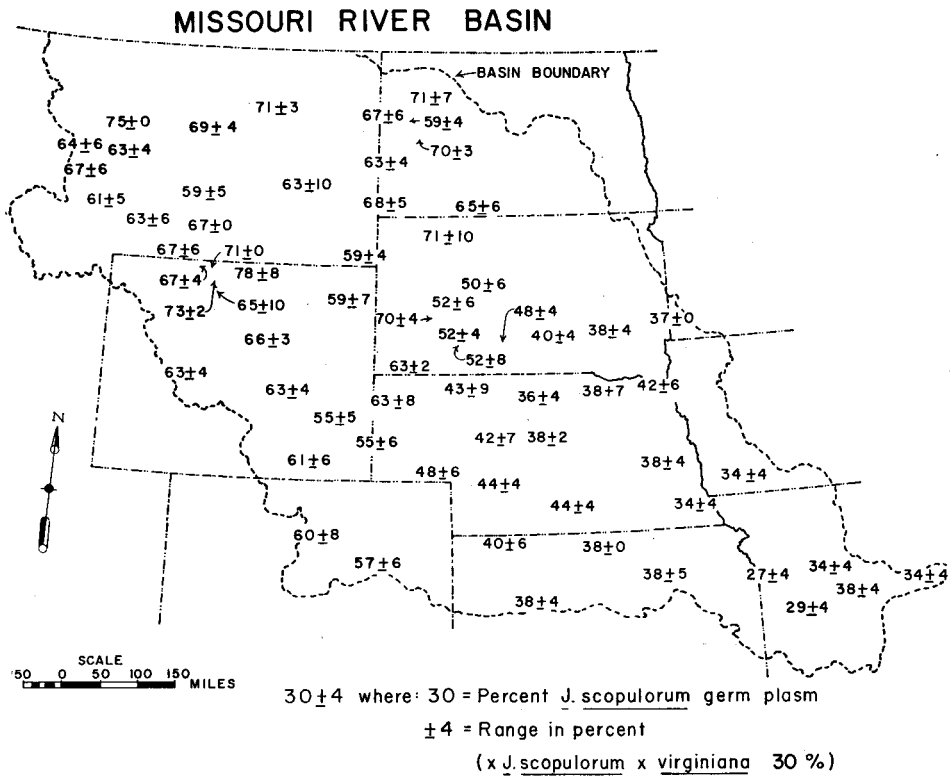


FIG. 13.—Geographic array of percent *J. scopulorum* germ plasm in *Juniperus* throughout the Missouri River Basin.

interpretation of the data—that is, the conversion of the hybrid index values at each collection site to a percentage germ plasm value. This analysis was selected because it represented a compromise between sample size (number of trees) and number of characters. Since the characters were morphological and, for the most part, measurable with readily available equipment, it would be feasible for other interested persons to compute hybrid index values from the characters used here and to determine the approximate composition of any sample they analyzed (refer to Table 9 for determination of hybrid index from character data).

Since in the hybrid index frequency distribution (Figs. 9a and 9b) the *J. virginiana* characters were assigned a value of 0, then an extreme specimen of *J. virginiana* would express a potential hybrid index of 0. And since the maximum hybrid index for *J. scopulorum* would have a value of 96 (adjusted to 100 for percentage purposes) the hybrid index represents, in effect, the percentage of *J. scopu-*

lorum germ plasm in the sample—*J. virginiana* with 0%, *J. scopulorum* with 100%. The adjustment to a percentage basis was made as follows:

Hybrid Index Value					
0–10	11–30	31–50	51–70	71–90	91–100
–	+1%	+2%	+3%	+4%	–

These percentages plus the ranges are arrayed geographically in Figure 13. (A collection site with a value of 30 ± 4, for example, would indicate that this sample of trees contained approximately 30% *J. scopulorum* germ plasm and 70% *J. virginiana* germ plasm. For the sake of convenience, a simple method of naming such individuals might be as follows:

x *J. scopulorum* x *virginiana* 30%

In each such a name, *J. scopulorum* would be listed first in accordance with Article 31(1) of the International Code of Botanical Nomenclature; and the percentage value would always refer to the germ plasm percentage of the first named species. Listing of the *J. virginiana* percentage in this scheme would be redundant. Other ways of expressing this hybrid relationship might be as follows:

x *J. scopulorum* (30%) x *virginiana* (70%)

or

x *J. scopulorum* x *virginiana* (30–70)

Conclusions

Data derived from this study of interrelationships between two arborescent species of *Juniperus* (*J. scopulorum* Sarg. and *J. virginiana* L.) throughout the Missouri River Basin are believed to be consistent and strong enough to justify the following conclusions.

The entire *Juniperus* population within the Basin is apparently of hybrid derivation with neither of the extreme parental types being found. There is a trend of increasing hybrid index values (also percentage germ plasm values) from southeast to northwest over the Basin from the reported range of *J. virginiana* to and into the reported range of *J. scopulorum*. This condition may be the result of bilateral introgression between the two species. There was, however, a strong tendency toward bimodality within the population as demonstrated by the presence of two distributions in each of the three hybrid indices. This indicated the presence of two different species—*J. scopulorum* and *J. virginiana*.

There is an apparent zone of hybrid values, comparable to an F_1 type, between the two species as also shown in the hybrid indices. This zone of intermediacy was demonstrated to be along and adjacent to a line extending northward from extreme western Kansas, into western Nebraska, diagonally northeastward through western South Dakota and the Badlands, then northward into central North Dakota. The magnitude of increasing hybrid index values toward *J. scopulorum* on the west side of the F_1 zone generally increases more rapidly than do the values decrease toward *J. virginiana* to the east of the zone. This is to be expected, since this zone parallels the geographic region of rapid transition from a gradual elevational rise across the Plains to an abrupt rise in topography leading into the Black Hills and the Rocky Mountains. Hence, the total of all the environmental factors becomes more rigorous in its selection of genotypes approaching *J. scopulorum*.

There is apparently an influence of *J. scopulorum* germ plasm eastward along the Missouri River, through the Dakotas, into eastern Nebraska and Missouri, as indicated by the presence of larger hybrid values in certain parts of this region. This is probably due,

in part, to the transport of *J. scopulorum* germ plasm down the Missouri River and, in part, to the proximity in the Dakotas of an environmental regime which is more rigorous in its selection of *J. scopulorum* genotypes.

The presence of distinct, but yet incomplete, bimodality and the absence of either extreme parental type indicates that introgression between the species has been underway for a considerable period of time but not long enough to merge completely the two populations. However, in view of the rather abrupt change from one environmental regime to another along the F_1 zone, complete merging of the two species may never be completed.

There is apparently introgressive hybridization involving a third species (*J. horizontalis* Moench.) into the predominantly *J. scopulorum* population in Montana and northern Wyoming. This is shown, in part, by the widespread presence of depressed individuals and an increase in the magnitude of the variation in the hybrid indices toward the northwest. Also, the wide range of environmental systems present throughout this vast and varied topographic area along with the isolated nature of many of the stands (Fig. 1) undoubtedly accounts for much of the variation demonstrated within the predominantly *J. scopulorum* population.

Infrared analysis of biological material may be a desirable source of relationship evidence as demonstrated by the high correlation of quantitative chemical data derived from IR spectra of plant lipid extracts with morphological data and the precision inherent in the IR technique. However, chemical data derived from IR analysis seem to be no more reliable for positive identification of parental species or of their hybrids that is any other single morphological character.

The data which have been presented can certainly be interpreted as evidence for the occurrence of introgressive hybridization between *J. scopulorum* and *J. virginiana*. However, there is another possible interpretation which, in the view of the author, is perhaps even more tenable.

One serious argument against the introgressive interpretation is the problem of initiation of introgression. This would demand the production of an F_1 from parental types which are some thousand miles apart, the establishment of this F_1, and subsequent backcrossing to both parents. As can be seen in Figures 8, 10, 12, and 13 the sites suitable for F_1 populations are certainly toward the western end of the *J. virginiana* range. In short, the original migration westward of the supposedly near genetically homogeneous *J. virginiana* germ plasm forms the crux of the problem.

As an alternative interpretation, it would seem that because of the greater diversity of the junipers in western North America, that *J. virginiana* was at some time derived from this area. It seems possible that with the inherent variability in the germ plasm ancestral to both *J. scopulorum* and *J. virginiana*, that propagules could flourish in sites toward the east. This could have initiated an eastward migration—propagule by propagule—which through mutation and selection eventually became what we now recognize as *J. virginiana.*

The present study area thus could represent the remnants of one such migration route—such as, along the Missouri and Platte River drainages. Since these propagules still carry a moderate amount of *J. scopulorum* germ plasm, recombination would permit offspring to inhabit a more *J. scopulorum*-type environment. This would explain the higher hybrid indices in the more rugged Appalachian areas as contrasted with those of the lower Missouri and Mississippi Valleys. Thus, rather than being considered as an introgressive series, the juniper population can alternatively be interpreted as a divergent series in which complete separation has yet to be attained.

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Appendix

Data sheets, IBM cards and programs, infrared spectra, photographs, illustrations, and other information used in the preparation of this study are on file in the Lincoln Field Unit of the Rocky Mountain Forest and Range Experiment Station, Lincoln, Nebraska, under study number FS-RM-1501.20.

A set of plant specimens has been deposited in the herbarium of the University of Nebraska. Duplicate sets have been filed with the herbaria of the University of Kansas, the Missouri Botanical Gardens, and the United States National Arboretum.

Quantitative Differences in Extractable Lipids Yield Taxonomic Character Data in *Juniperus*[1]

By

David F. Van Haverbeke[2], Charles Y. Sullivan[3], and John F. Davidson[4]

ABSTRACT

This paper describes a quantitative technique for obtaining a crude lipid extract from *Juniperus* cone pulp and its analysis by infrared (IR) spectroscopy to obtain chemical character data. Correlation was found between the chemical characters and morphological characters. Sampling extended across more than 1,200 miles throughout the Missouri River Basin from the range of *Juniperus scopulorum* Sarg. to that of *J. virginiana* L. The study suggests the desirability of this technique as a source of relationship evidence in taxonomic research.

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INTRODUCTION AND LITERATURE REVIEW

This paper reports on a quantitative technique for obtaining a crude lipid extract from *Juniperus* cone pulp, the analysis of this extract by infrared (IR) spectroscopy to obtain chemical character data, and suggests this technique might provide useful relationship evidence in taxonomic research. While infrared has been used qualitatively to isolate, identify, and characterize specific compounds in discretely different taxa, no reference could be found regarding its quantitative utilization as supporting evidence in a taxonomic analysis of plant populations over a broad area.

Erdtman (1956) emphasized that, taxonomically, the most important chemical substances are not those involved in primary metabolic processes, but rather those which are relatively stable by-products of their biological environment. The plant wax hydrocarbons, which are included in this category, lend themselves to rapid isolation and quantitative analysis. Erdtman also stressed that the complexity of the plant wax hydrocarbons is a positive advantage in providing a taxonomic fingerprint. Eglinton *et al.* (1962a) examined leaf waxes of a compact group of closely related genera of the sub-family *Sempervivoideae* of the family *Crassulaceae*, and reported that comparisons might confirm relationships between closely related species. These authors caution, however, that similar species sometimes have widely different patterns, and that there is only a rough parallelism of hydrocarbon pattern and botanical classification. Eglinton *et al.* (1962b) studied the hydrocarbon fractions of certain New Zealand plants and suggested their possible taxonomic implications.

Erdtman (1963) stated that chemical characteristics are genetically controlled, and have the advantage over morphological characters in that they can be very exactly described in terms of definite chemical structure and configuration formulae. Heslop-Harrison (1963) believed that if chemical data are used to refine the natural system, the potentialities of chemical data must be assessed (correlated) against all other data. Bate-Smith (1963) and Walters (1963) also stressed the need for chemical data to be correlated with morphological characters if they are to be of use to the taxonomist, who must rely mainly on visual characteristics for the recognition and

classification of natural forms. Such correlations have been made in the present study.

MATERIALS AND METHODS

In the fall of 1965 a study was started to determine the extent of variation in gross morphological, foliage, cone, and seed characters of Rocky Mountain juniper (*Juniperus scopulorum* Sargent) and eastern redcedar (*Juniperus virginiana* L.) throughout the Missouri River Basin. The objective was to clarify some of the taxonomic interrelationships between these two species for subsequent use in tree improvement programs in the Great Plains. Ten trees were sampled from about 70 native stands of *Juniperus* throughout the Basin.[5] This drainage system, encompassing most of the Central and Northern Great Plains, contains portions of the botanical ranges of the two species under investigation—Rocky Mountain juniper in the western half and eastern redcedar in the eastern half. Fassett (1944) reported the presence of hybrid swarms between these species within the area where the species ranges were reported to overlap or meet.

There are many references to positive correlation between accumulation of various lipids and increased cold hardiness (Levitt, 1956). Since the range of *J. scopulorum* extends further into the colder regions of the Missouri River Basin than does *J. virginiana*, it seemed desirable to investigate some of the chemical characteristics that have been related to cold hardiness. The hope was to obtain correlation of chemical character data with morphological character data.

Crude lipid extracts of cone pulp were prepared from mature cones collected at many of the sites by the following procedure:

1. Seed extracted from frozen cone lots,
2. Three or four grams of cone pulp oven-dried for 18 hrs. at 65°C.,
3. Dried pulp pulverized in mortar with pestle,
4. One gram sample of pulp placed in 15 ml centrifuge tube,
5. Five ml CCl_4 added,
6. Pulp and CCl_4 shaken two minutes with an automatic shaker,

5. Herbarium specimens have been deposited in the University of Nebraska Herbarium. Duplicate sets have been filed with the herbaria of the University of Kansas, the Missouri Botanical Gardens, and the United States National Arboretum.

7. Tube covered with Saran wrap and allowed to stand overnight at room temperature,
8. Sample reshaken two minutes,
9. Sample centrifuged in conical tubes at 1000 x g for 15 min.,
10. Three ml extract drawn off with needle and syringe,
11. Extract filtered through fritted glass filter,
12. Extract dried to less than 0.5 ml over air jet,
13. Extract brought back accurately to 0.5 ml by adding CCl_4,
14. Barium fluoride IR cell (.05 ml pathway) filled with extract,
15. Spectra run on Beckman IR-10 Infrared Spectrophotometer.

Direct extractions were made with CCl_4 because of its desirability as an infrared medium. The results were compared with those from extracts made with both petroleum ether and ethyl ether, which were dried and redissolved in CCl_4. Spectra were nearly identical. Samples were repeated for cone lots at three locations (total of 14 trees) to test for precision of technique.

RESULTS AND DISCUSSION

Spectra

It was originally thought that distinct qualitative differences in the crude lipid extracts might be detected when examined by infrared spectroscopy. However, after a number of these extracts were run, it became apparent that the major differences in the spectra were quantitative; that is, the respective absorption peaks occurred at the same wavenumber—mainly the magnitude of expression varied. This agrees with Flück (1963), who stated that chemical nonuniformity in a given taxon is mainly quantitative. Spectra of a selected group of individual trees illustrate the pattern of increasing infrared absorption in a southeast-northwest direction within the study area (Fig. 1).

There was a tendency, however, for the conformation of portions of the spectra in this investigation to vary in a fairly consistent pattern. For instance, proceeding westward and northward from Missouri, the absorption of wavenumber 2825 cm^{-1} tends to decrease relative to the absorption at wavenumber 2915 cm^{-1} (Fig. 1). Also, the conformation of the spectra in the region of wavenumber 1700 cm^{-1} tends to shift to the right in eastern trees and to become broader at the base of the peak in the more westerly trees. Such differences may be helpful in analyzing other plant populations.

On the basis of absorption peaks, the compounds were placed in broad chemical classes and further divided into saponifiable and nonsaponifiable groups. Extracts were saponified by refluxing for

4 hours in 10% KOH in 95% ethanol, extracted with petroleum ether, dried, and redissolved in CCl_4. The major peaks associated with the region around wavenumbers 2915, 1450, and 1385 cm^{-1} are characteristic of long chain carbon-hydrogen bond stretching and bending modes (Fig. 1). The peak around 1700 cm^{-1} indicates a carbonyl radical. It may be in an aldehyde, ketone, ester, or acid. The main peak in this region is found at 1695 cm^{-1} (ketone) with shoulders at 1715 and 1735 cm^{-1} (ester). Attempts to saponify the extracts resulted in saponification at 1695 and 1735 cm^{-1}, partial saponification at 1715 cm^{-1}, and absence of saponification at 2915, 1450, and 1385 cm^{-1}.

While it might be desirable to identify these compounds accurately, for the present purpose this was not considered essential. Peaks in the spectra are due to specific functional groups which may be shared in common by a number of compounds. A peak, however, is due to perhaps a single, unique enzymatic action (e.g., hydroxylation of phenolics by oxidases). This could lead to a situation where separation of the compounds contributing to the IR peaks may or may not show a difference in the taxonomic group for any one compound. What has actually been measured may be a general enzymatic reaction involving a number of different substrates which may vary between the species because of other biochemical differences. It was, therefore, decided not to undertake complete analysis of the compounds at this time.

Infrared Absorption

Cone pulp extracts of over 150 sample trees showed increasing absorption at a given wavenumber in a southeast to northwest pattern over the study area; that is, from the range of *J. virginiana* to and into the range of *J. scopulorum* (Fig. 2). Optical density values were calculated at 14 wavenumbers across the spectra (2930, 2915, 2835, 2825, 1735, 1725, 1715, 1695, 1685, 1465, 1450, 1435, 1385 and 1365 cm^{-1}). Mean optical densities for the peak at wavenumber 2915 cm^{-1} are shown in Figure 2 for trees sampled at 51 locations. The remaining peaks and shoulders were highly correlated with the peak at 2915 cm^{-1}, but the absorption was less. Optical densities for individual trees ranged from a low of .40 in Missouri to 1.7 in North and South Dakota. Spectra from repeated samples of cone pulp from the same tree were precise within a range of $\pm$ 3%.

While plants do not operate in terms of means, even the average absorption values in Figure 2 show a gradient from the region of

J. scopulorum (northwest) to that of *J. virginiana* (southeast). Although the above refers to absorption only at wavenumber 2915 cm^{-1}, it can be seen from Figure 1 that plotting of optical density values at other wavenumbers would yield comparable results. These same trees are included in a larger sample in which 24 morphological characters were analyzed (Fig. 3). These data show that the trend in morphology is comparable to that shown in the IR absorption (Fig. 2).

Correlations

Trends of increasing cone volume and length of leaf-scale resin gland were found in a southeast-northwest pattern in the study area. These data, expressed in terms of cone volume and leaf-scale resin gland length, were compared statistically against optical density values at wavenumber 2915 cm^{-1} (Figs. 4 and 5). Correlation coefficients (r) of .405 and .568, both significant at the 1% level, were obtained.

This apparent correlation between morphological and chemical characters was further confirmed by computing the correlation coefficients between each of the 24 morphological and 14 optical density (chemical) characters (See Table 3). The fact that 336 consistent and, for the most part, individually significant correlations were obtained essentially removes the element of chance as far as significance is concerned. As the morphology is controlled genetically (Ownbey and Aase, 1955, as in Benson, 1962, p. 276); it follows that the biochemistry is also under genetic control (Erdtman, 1963). This leaves to the environment primarily the role of selecting appropriate germ plasm.

Some optical density values higher than what might be expected (reflected in higher mean values) were recorded in samples from sites in eastern Nebraska and Missouri along the Missouri River (Fig. 2). These higher values support the hypothesis (Van Haverbeke, 1965) that there may have been a movement or an extension of *J. scopulorum* germ plasm, via floating cones and seeds, down the major drainage system of the Missouri River from the Dakotas. Propagules whose genetic range of tolerances have remained within the extremes of the environmental stresses in eastern Nebraska and Missouri apparently have become established and have injected an appreciable amount of *J. scopulorum* germ plasm into the subsequent local populations. Morphological characters resembling the western species were also detected at these locations.

The eastern part of the reported Rocky Mountain juniper range extends diagonally northeastward from eastern Colorado across

western Nebraska, the Badlands of South Dakota, and northward to the Missouri River through the Dakotas (Fowells, 1965). Thus, the occurrence of these higher optical density values tending toward *J. scopulorum*, further to the east, indicates the presence of an environment which has selected *J. scopulorum* type plants for survival. Fassett (1944) reported the presence of hybrid trees as far east as Chamberlain, South Dakota.

The large tributaries of the upper Missouri River may also offer a more extensive collecting and transporting system for juniper cones and seeds than do the tributaries of the Platte River. Whatever the cause, trees along the Missouri River in eastern Nebraska and Missouri seem to possess a higher percentage of *J. scopulorum* characteristics than do the trees along the Platte River in central and eastern Nebraska.

Extracts from certain trees in Wyoming, Montana, and the western Dakotas showed optical density values somewhat lower than those from most other western locations (Fig. 2). Analysis of morphological data on these trees suggested the influence of a third species, (*J. horizontalis Moench.*), upon the *J. scopulorum* population. Infrared spectra run on a limited sample of *J. horizontalis* cone pulp revealed low optical densities of 0.62 to 0.76. This, in part, may explain the low optical density values recorded in otherwise *J. scopulorum* areas.

Means, Ranges and Coefficients of Variation

In a tree breeding program it is desirable to know as much about the genotypes of individual trees as possible. To gain some insight about the value of infrared analysis for evaluating individual trees for species identification or degrees of hybridity, mean optical densities, ranges of the means, and coefficients of variation were calculated (Fig. 6). These samples represent the range of optical densities from four or more trees at a site. These data clearly reflect the directional cline of increasing optical densities. Mean optical densities for locations and the ranges of individual tree values show considerable overlap, however. Coefficients of variation are generally acceptable, with a range of 9 to 31 percent (Fig. 6).

While this study is considered to be exploratory in nature, and while these chemical data seem to be no more reliable for positive identification of parental species or of their hybrids than is any other single morphological character, they do provide evidence that infrared analysis of biological materials can supplement and thereby strengthen a population analysis based on conventional morphological characters.

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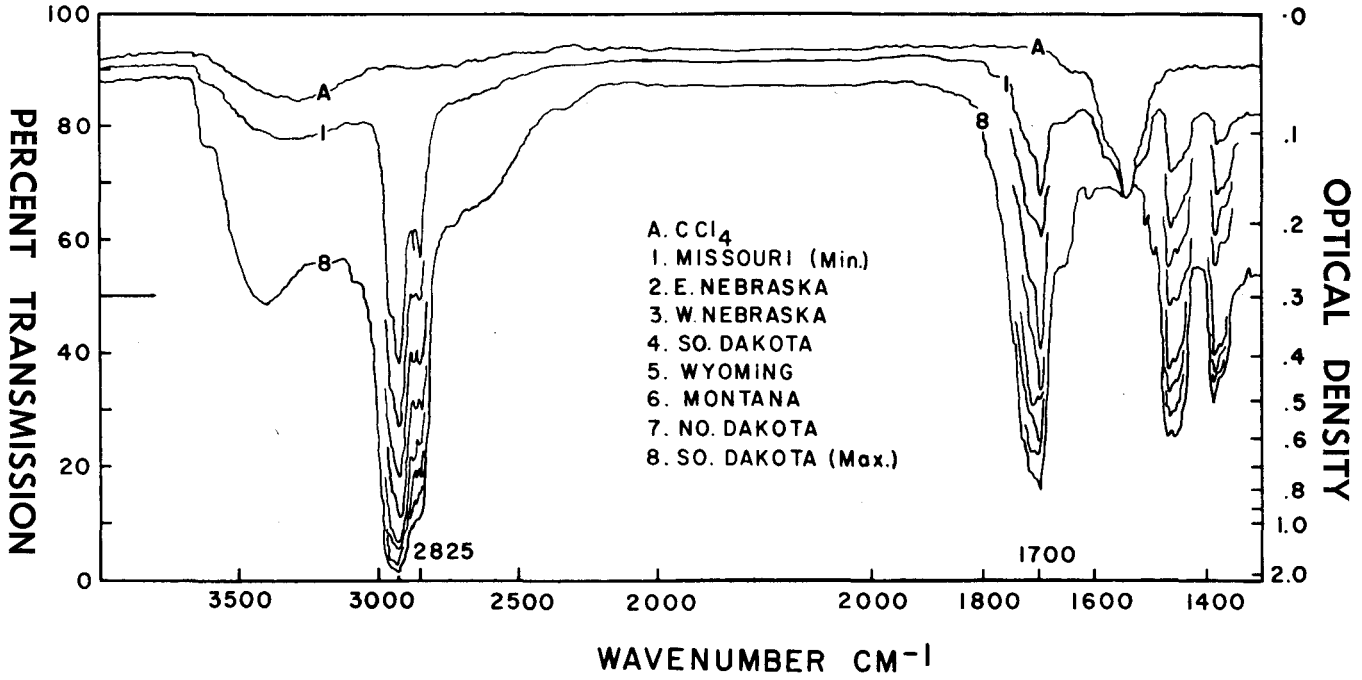

FIG. 1.—Infrared spectra of a selected group of juniper trees illustrating quantitative differences in optical densities from southeast to northwest throughout the study area. Note differences in peak conformation in the vicinity of wavenumbers 2825 cm^{-1} and 1700 cm^{-1}.

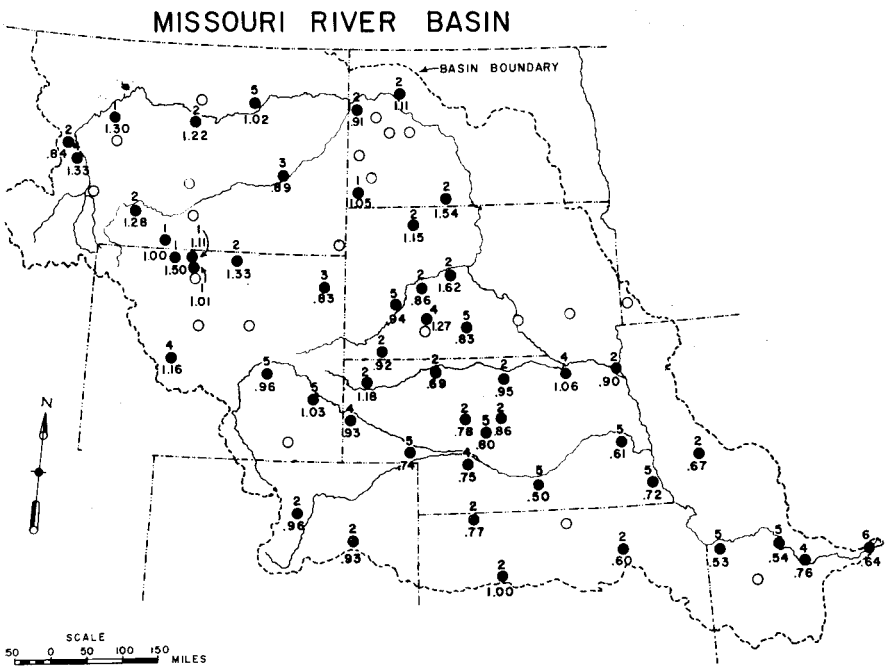

FIG. 2.—Collection sites used in the infrared analysis (black circles) and other collection sites (white circles). Top figure indicates number of trees sampled and bottom figure indicates mean optical density at wavenumber 2915 cm^{-1} for the group. (n = 152).

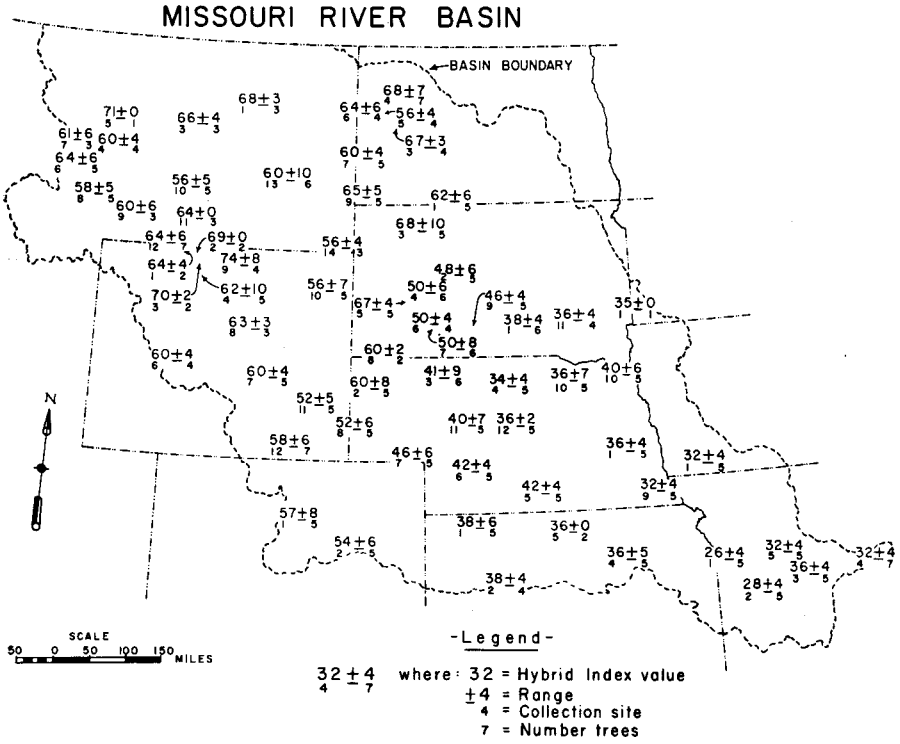

FIG. 3.—Geographic array of hybrid index values derived from 343 *Juniperus* trees and utilizing 24 gross morphological, foliage, and cone and seed characters.

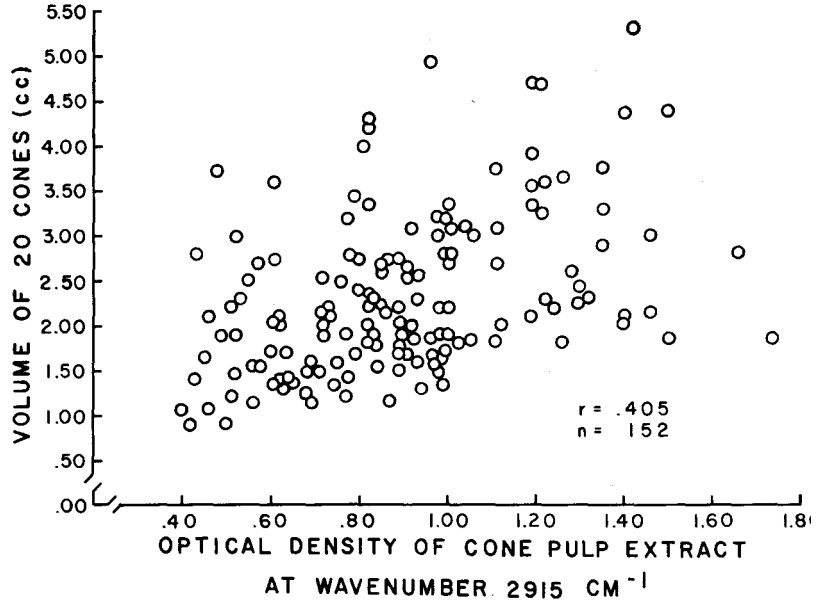

FIG. 4.—Relationship between optical density of cone pulp extract at wavenumber 2915 cm^{-1} and volume of whole cones for juniper trees sampled throughout the Missouri River Basin. (Correlation coefficient significant at 1% level.)

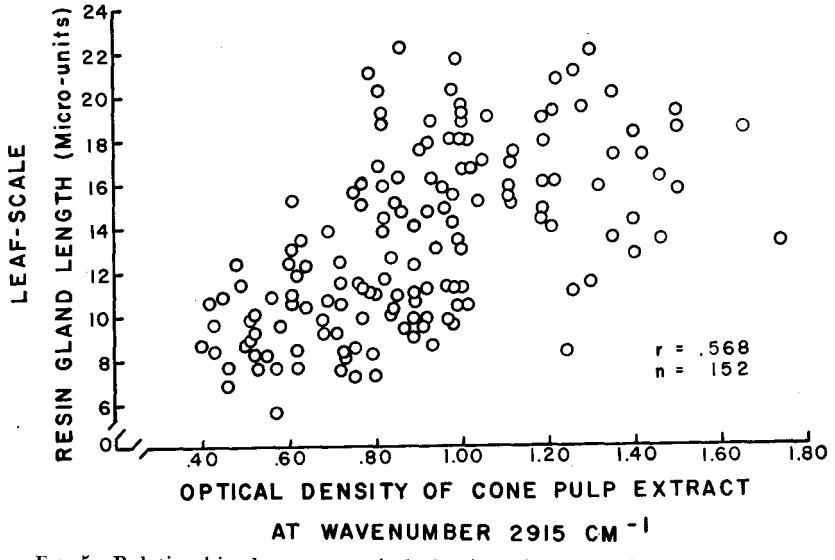

FIG. 5.—Relationship between optical density of cone pulp extract at wavenumber 2915 cm^{-1} and length of leaf-scale resin gland for juniper trees sampled throughout the Missouri River Basin. (Correlation coefficient significant at 1% level; 30 micro-units = 1 mm.)

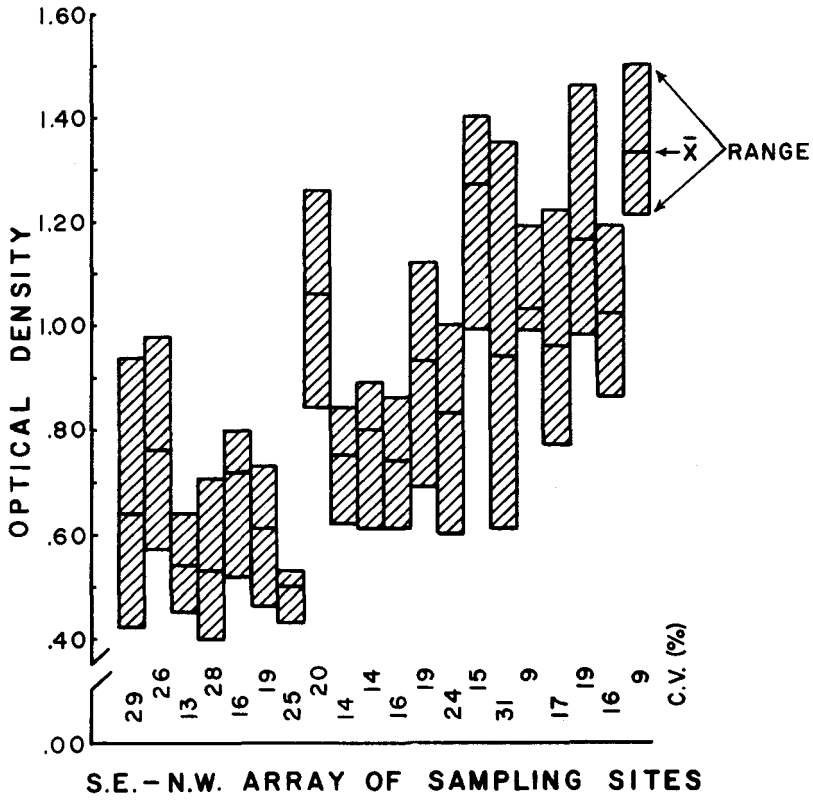

FIG. 6.—Mean optical densities, ranges, and coefficients of variation for juniper trees in a southeast–northwest array. (Locations contain 4 or more trees with IR spectra.)

A NOTE ABOUT THE AUTHOR

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